VOLUME XXXV JULY, 1956 NUMBER 7

The Journal of Clinical Investigation

EDITED FOR THE AMERICAN SOCIETY FOR CLINICAL INVESTIGATION

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Printed by the Lancaster Press, Inc., Prince and Lemon Sts., Lancaster, Pa. Published by the American Society for Clinical Investigation. Publication Office, 622 West 168th St., New York 32, N. Y.

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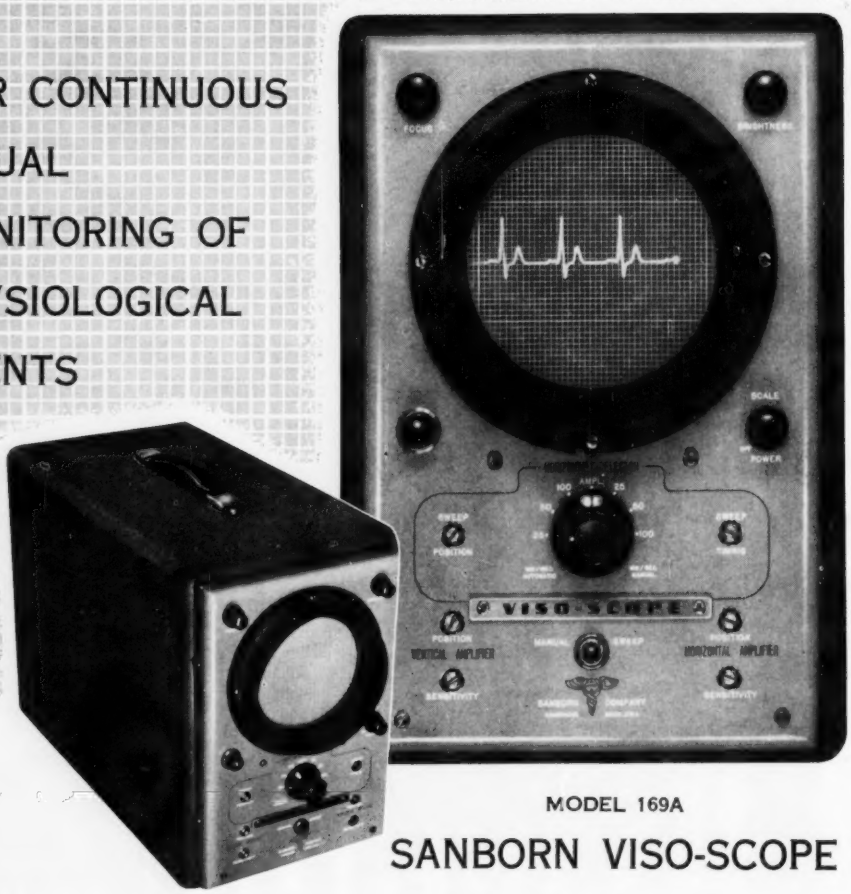

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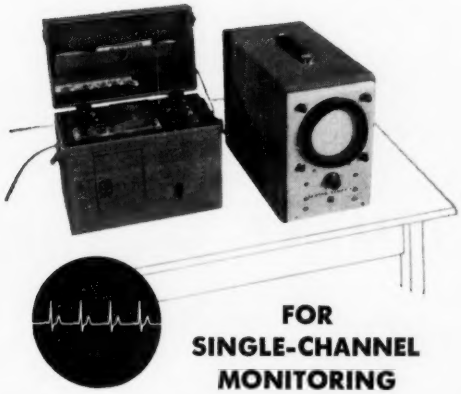

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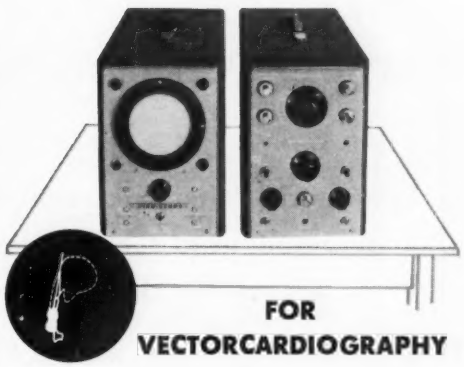

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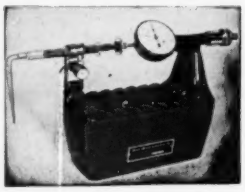

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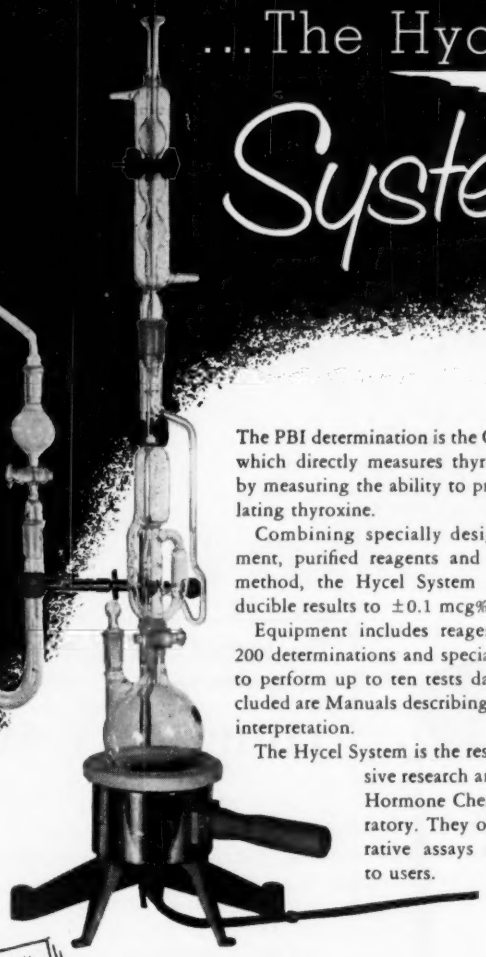

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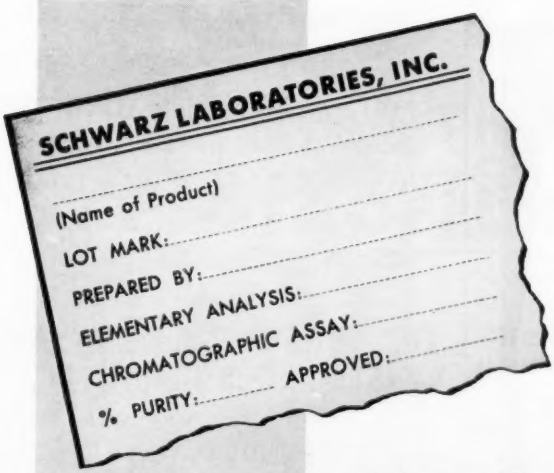

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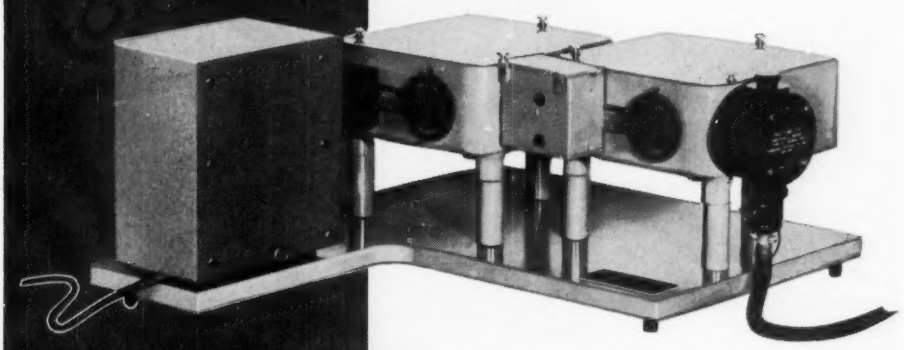

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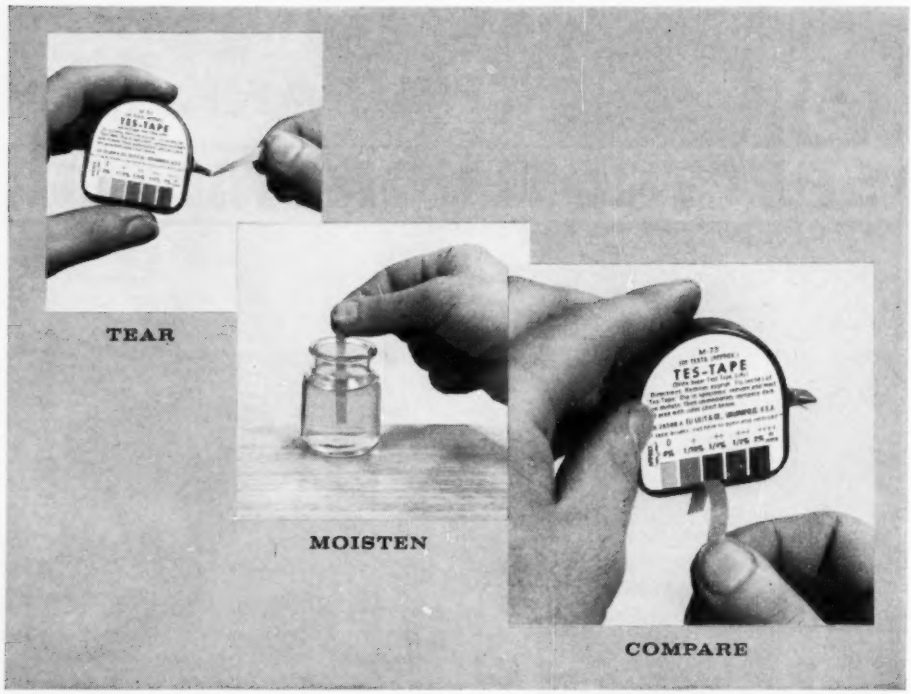

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THE EFFECT OF VARIATIONS IN SOLUTE EXCRETION AND GLOMERULAR FILTRATION ON WATER DIURESIS [1]

By CHARLES R. KLEEMAN, FRANKLIN H. EPSTEIN, AND COLIN WHITE

(From the Department of Internal Medicine and the Biometry Section, School of Public Health, Yale University School of Medicine, New Haven, Conn.)

(Submitted for publication December 16, 1955; accepted March 15, 1956)

A sustained positive load of water administered to a normal subject results in maximal water diuresis associated with a minimal concentration of solutes in the urine. The term "physiological diabetes insipidus" has been applied to this situation, because under these circumstances, the release of antidiuretic hormone from the posterior pituitary is inhibited and as a consequence, that part of the water of glomerular filtrate that is normally reabsorbed under the influence of this hormone is excreted. During sustained water diuresis, therefore, variations in urinary flow, urinary osmolarity, and "free water clearance" (C_{H_2O}) (1) might be ascribed to factors other than antidiuretic hormone.

In the present study, the effect of variations within the physiological range of solute load, dietary composition and endogenous creatinine clearance upon these parameters were investigated in normal subjects in a state of "physiological diabetes insipidus," in whom the level of circulating antidiuretic hormone was presumably at a minimum. The results indicate that both glomerular filtration rate (endogenous creatinine clearance) and solute load have important and statistically separable effects on the characteristics of water diuresis.

MATERIALS AND METHODS

Twenty-nine experiments were performed on fourteen healthy young male medical students and physicians. After a light breakfast at 7:00 A.M. the experiment was begun at about 8:30 A.M. and varied in length from 3-½ to 5-½ hours. The subjects remained in the semi-recumbent position throughout, getting up only to void.

In twelve paired experiments on six subjects a "high-solute diet" or a "low-solute diet" was ingested for three days prior to the experiment. The "high-solute diet" contained approximately 150 grams of proteins and 10 to 15 grams of salt; the "low-solute diet" contained less than 25 grams of protein and 5 grams of salt. In all other experiments diet was uncontrolled. Subjects drank one liter of water upon reclining. A sustained positive balance of one liter was maintained throughout the rest of the experiment by oral ingestion of water and by the infusion of 2.5 per cent fructose in water at rates below that necessary to produce glycosuria when tested with Benedict's solution.

Sodium, potassium and chloride in serum and urine were measured by methods previously described (2, 3). Creatinine in serum and urine was determined by a modification of the method of Hare (4). Endogenous creatinine clearance was used to approximate glomerular filtration rate. Urea in serum and urine was measured by a micro-diffusion technique (5); and the osmolarity of serum and urine was measured by freezing point depression using a Fiske osmometer. These results are obtained in milliosmols per kilogram of water. Free water clearance (C_{H_2O}) was calculated as the difference between urinary flow V and osmolar clearance: $C_{H_2O} = V - \frac{UosmV}{Posm}$ (1), where V = urinary flow (cc. per min); Posm = osmolar concentration of plasma (mOsm. per Kg.); Uosm = osmolar concentration of urine (mOsm. per Kg.).

RESULTS

I. Effect of variations in solute excretion upon water diuresis

a. *Maximal urinary flow* (*Table I*): Maximal urinary flow varied from 9.7 to 23.7 cc. per minute per 1.73 m^2, and was directly related to the concomitant rate of excretion of solute (Figure 1 and Table IIa). Using the statistical technique of multiple regression analysis (see below), it was apparent that variations in the rate of excretion of either electrolytes ([Na + K] 2) or non electrolytes (total urinary osmols − [Na + K]2) had no significant separate effect over and above their contribution to the total solute excretion (Table III).

b. *Minimal urinary osmolarity* (*Table I*): At the height of water diuresis urinary osmolarity varied from 40 to 85 milliosmols per Kg. with a mean of 62.5 milliosmols per Kg. This minimal urinary concentration was higher in the same sub-

[1] Supported by United States Public Health Service Grants A-625 and H-834.

TABLE I

Tabulation of variables *

Subject	Surface area M^2	G.F.R. *cc./min.*	Solute load *μOs./min.*	Urine flow *cc./min.*	$[Na+K]_U$ *μEq./min.*	Urea *μM/min.*	Minimal urinary osmolarity *mOs./Kg.*	Minimal serum osmolarity *mOs./Kg.*	C_{H_2O} *cc./min.*
R. S.	1.85		1,517	23.4			69	285	
R. S.	1.85		1,200	15.9			68		
K. S.	2.00	104	638	10.0			65	282	7.8
M. R.	1.90	121	715	11.2			59	279	8.8
F. E.	1.79	134	1,014	17.0			62	283	13.4
E. S.	2.00	128	1,207	20.2	803		63	284	16.0
E. S.	2.00	117	753	15.7	364		49	275	13.0
W. H.	2.07	138	1,100	20.4	687		68	280	15.1
D. B.	2.13	102	933	13.0			66	277	9.3
N. U.	1.92	144	839	18.0	483		50	274	14.8
M. R.	1.90	121	828	11.3	437		67	282	8.2
P. H.	1.84	126	840	11.6	354		77	284	8.5
M. R.	1.90	102	1,235	12.6	630		68	302	8.6
C. K.	1.88	129	513	9.7	311	192	62	280	6.9
C. K.	1.88	129	658	10.6	258	402	71	276	7.6
F. K.	2.07	99	690	11.5	363	242	57	287	8.9
M. S.	1.98	135	1,185	16.8	248	785	66	282	12.7
C. K.	1.88	112	626	11.0	490	194	63	278	8.5
C. K.	1.88	106	906	12.2	488	270	68	300	9.2
F. E.	1.79	144	1,177	21.2	390	734	58	285	15.5
F. E.	1.79	130	786	16.4	609	200	52	282	13.4
E. L.	2.05	170	984	14.9	287	514	54	274	14.3
E. L.	2.05	135	620	14.0	285	236	41	276	12.2
M. S.	1.98	124	489	13.0	194	221	40	270	11.2
D. K.	1.90	118	598	13.5	231	264	46	270	11.3
D. K.	1.90	130	2,000	23.7	1,965	290	85	270	16.1
M. S.	1.98	118	1,310	17.0	784		77	285	13.0
R. S.	1.85	122	1,620	20.1			80	280	14.4
F. E.	1.79	130	1,190	20.8	484	700	57	280	16.9

* All values corrected to 1.73 M^2 and they represent the mean of three to five periods at the height of the water diuresis.

TABLE II

Analyses of variances of the parameters of water diuresis

Source of variation*	Degrees of freedom	Sum of squares	Mean square	Variance ratio
(a) Urinary flow				
1) Multiple regression on X_1 and X_2	2	301.05		
Simple regression on X_2	1	254.58	254.58	52.40†
Extra effect due to X_1	1	46.47	46.47	9.56‡
2) Deviations from multiple regression	24	116.66	4.86	
3) Total	26	417.71		
(b) Minimal urinary osmolarity				
1) Multiple regression on X_1 and X_2	2	1,705.70		
Simple regression on X_2	1	1,405.40	1,405.40	21.26†
§Extra effect due to X_1	1	300.30	300.30	4.54§
2) Deviations from multiple regression	24	1,586.97	66.12	
3) Total	26	3,292.67		
(c) Free water clearance (C_{H_2O})				
1) Multiple regression on X_1 and X_2	2	131.22		
Simple regression on X_1	1	80.11	80.11	21.29†
Extra effect of X_2	1	51.11	51.11	13.58‡
2) Deviations from multiple regression	22	82.79	3.76	
3) Total	24	214.01		

* X_1 = glomerular filtration rate. X_2 = solute load.
† Significant ($P < 0.001$).
‡ Significant ($0.001 < P < 0.01$).
§ Significant ($P < 0.05$), as indicated in the text the regression in this case is negative.

TABLE III

Analysis of variance of urinary flow *

Source of variation†	Degrees of freedom	Sum of squares	Mean square	Variance ratio
1) Multiple regression of Y on X_1, X_2 and X_3	3	231.45		
Multiple regression on X_1 and X_2	2	230.29		
Extra effect due to X_3	1	1.16	1.16	0.19
Multiple regression on X_1 and X_3	2	192.01		
Extra effect due to X_2	1	39.44	39.44	6.38‡
Multiple regression on X_2 and X_3	2	197.46		
Extra effect due to X_1	1	33.99	33.99	5.50‡
2) Deviations from multiple regression on X_1, X_2 and X_3	18	111.33	6.19	1.00
3) Total	21	342.78		

* This analysis has been placed in a separate table because of the addition of the added dependent variable of electrolyte excretion $2(Na^+ + K^+)$ and the utilization of 22 rather than 27 experiments.
† Y—urinary flow. X_1 = glomerular filtration rate. X_2 = solute load. X_3 = electrolytes.
‡ Significant ($0.01 < P < 0.05$).

ject on a high-protein, high-salt diet than on a low-solute diet (Figure 2a) and its magnitude was directly correlated with the rate of solute excretion in the entire group of experiments (Figure 2b, Table IIb). There was no correlation between the osmolar concentration of serum and the minimal urinary osmolarity (Figure 3).

c. *Free water clearance* (C_{H_2O}) (*Table I*): C_{H_2O} varied from 6.8 to 16.9 cc. per minute. The increase in urinary flow accompanying an increase in dietary intake and urinary output of solutes consisted not only of an increase in osmolar clearance (Cosm) but also of a rise in C_{H_2O}. This correlation is most obvious in paired studies on the

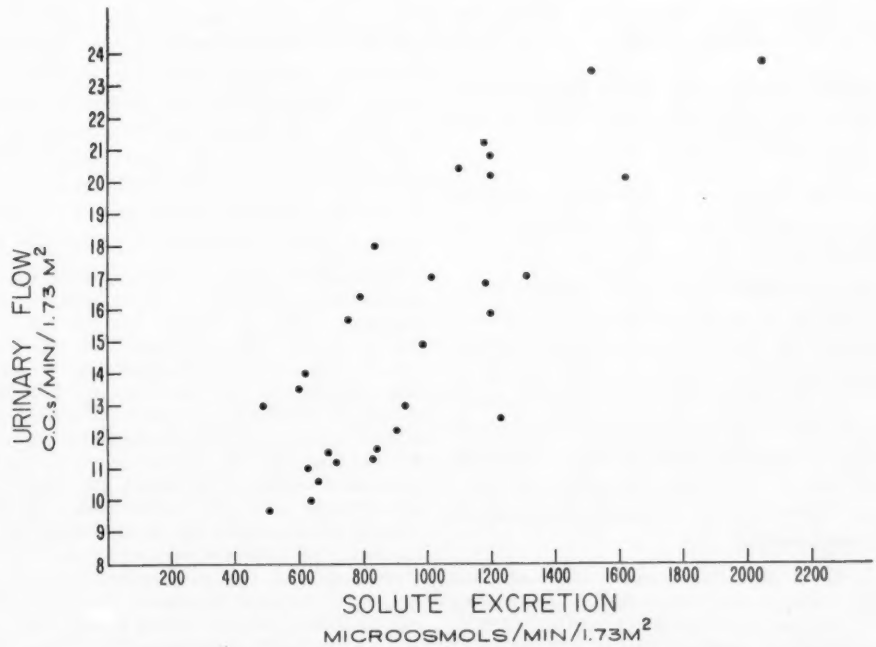

FIG. 1. THE RELATIONSHIP BETWEEN THE MAXIMAL URINARY FLOW AND THE RATE OF SOLUTE EXCRETION AT THE HEIGHT OF SUSTAINED WATER DIURESIS

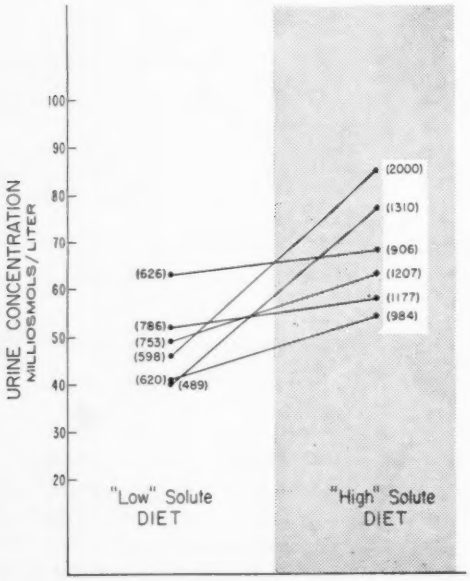

FIG. 2a. ILLUSTRATION OF THE EFFECT OF AN INCREASE IN THE RATE OF SOLUTE EXCRETION UPON THE MINIMAL URINARY OSMOLARITY IN PAIRED DIETARY EXPERIMENTS

The numbers in parentheses represent the rate of solute excretion in microosmols per minute.

same subject (Figure 4a) but is also apparent when all the data are plotted as in Figure 4b and treated statistically (Table IIc).

II. *Effect of variations in glomerular filtration rate (endogenous creatinine clearance) upon water diuresis*

Because it is difficult, in single experiments in human subjects, to produce variations in glomerular filtration rate without simultaneously altering the rate and pattern of solute excretion, the statistical method of multiple linear regression analysis (6) was resorted to in order to attempt to evaluate the effect of changes in endogenous creatinine clearance *per se* upon the parameters of water diuresis. The following is a brief description of the statistical method.

The effects of creatinine clearance and solute load were tested, using in turn, urinary flow, minimal urinary osmolarity and C_{H_2O} as the dependent variable. In principle the method is to subdivide the total variation found in the dependent variable into various meaningful components: (a) that due to the independent variables, namely solute load and creatinine clearance, and (b) a remainder, which is loosely classified as error, and is due to factors not included in the analysis as well as to imperfect reproducibility of the measurements.

The results from each experiment may be represented by a point in three-dimensional space with co-ordinates x_1, x_2 and y, where x_1 is the creatinine clearance rate, x_2 the solute load and y the urine flow. A plane may then be fitted by the method of least squares to the swarm of points. If this plane is effectively parallel to the plane containing x_1, x_2 then the independent variables are without detectable influence on the dependent variable. If the fitted plane is sloped in such a way that y changes significantly with either x_1, or x_2 or both then the appropriate independent variable is said to have an effect on y. The decision as to what change is regarded as significant must be made in the light of the "error" of the model used, that is the deviations between the values of y predicted from the fitted plane and the values actually observed. The tests of significance are formally carried out by means of the analysis of variance. The results are shown in Tables II and III.

a. *Maximal urinary flow (Table IIa)*: A preliminary examination by the method of simple linear regression showed that each of the factors, creatinine clearance and solute load, considered separately, was significantly related to the magnitude of urine flow.[2] The sum of squares accounted for by the regression on solute load alone was 254.58 and that accounted for by regression on creatinine clearance alone was smaller, namely 85.66. However, these two factors are themselves correlated and it was therefore necessary to decide whether creatinine clearance produced an effect over and above that attributed to solute load, or whether the effect apparently due to creatinine clearance arose simply from correlation of this

[2] It should be pointed out that in making this analysis we have assumed that the 29 sets of observations are independent, whereas, in fact, the data were collected from only 14 subjects. When a subject was examined on a second occasion, however, the conditions as regards diet were quite different from the first and it is possible that the changes thereby produced were so great as to make differences between subjects unimportant. This assumption has been tested by running an analysis on data in which each subject is represented only once. The results obtained completely confirm those reported below with the single exception that the effect of glomerular filtration rate on minimal urinary osmolarity, though qualitatively identical with that reported, does not rise to the level of statistical significance. In view of this general confirmation it is considered reasonable to treat the observations on the same subject as if they were independent, though anyone who preferred not to do this would arrive at essentially the same conclusions.

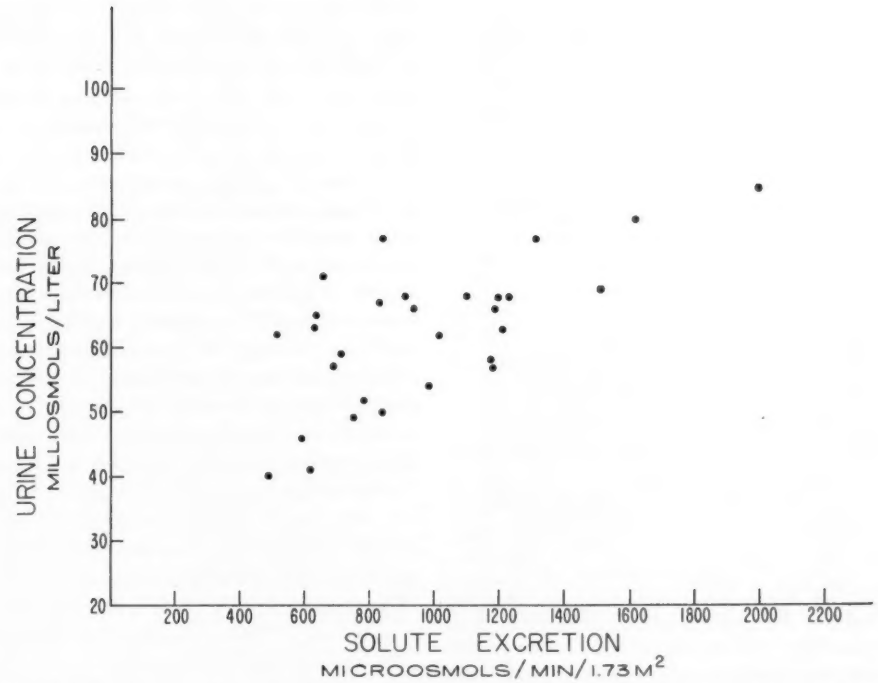

FIG. 2b. THE RELATIONSHIP BETWEEN THE MINIMAL URINARY OSMOLARITY AND THE RATE OF SOLUTE EXCRETION AT THE HEIGHT OF SUSTAINED WATER DIURESIS

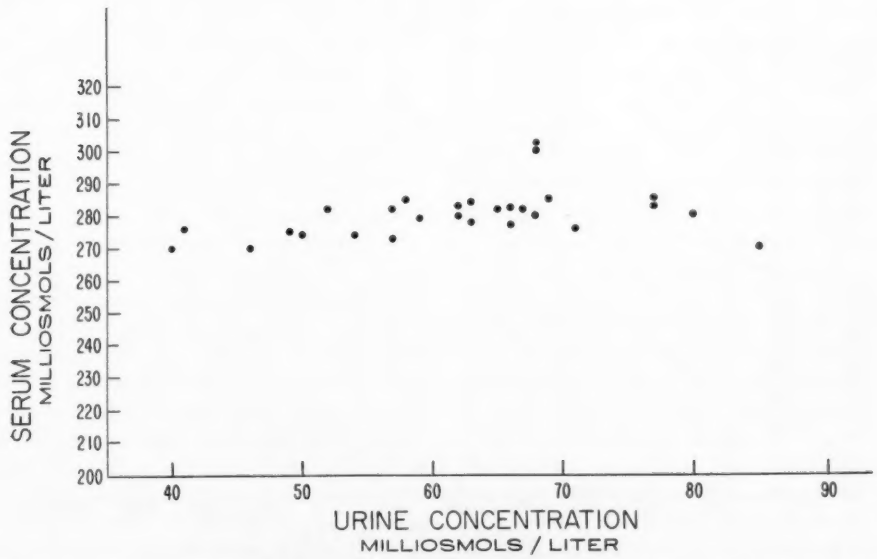

FIG. 3. ILLUSTRATION OF THE LACK OF CORRELATION BETWEEN THE MINIMAL URINARY OSMOLARITY AND THE OSMOLAR CONCENTRATION OF THE SERUM AT THE HEIGHT OF THE DIURESIS

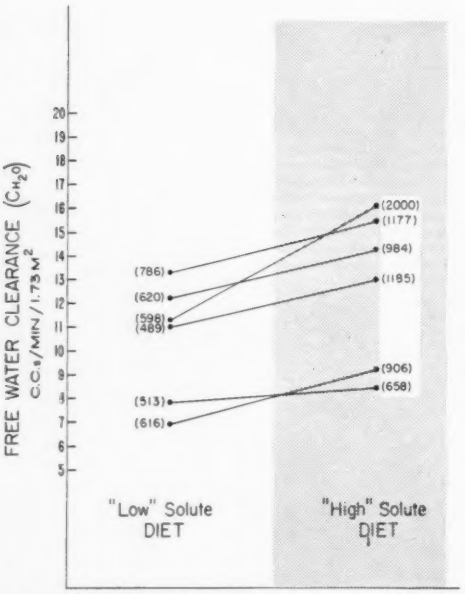

FIG. 4a. ILLUSTRATION OF THE EFFECT OF AN INCREASE IN THE RATE OF SOLUTE EXCRETION UPON THE FREE WATER CLEARANCE IN PAIRED DIETARY EXPERIMENTS

The numbers in parentheses represent the rate of solute excretion in microosmols per minute.

factor with solute load. The tests are given in Table IIa and reveal that creatinine clearance, in addition to any effect produced by virtue of its correlation with solute load, is also directly and independently correlated with maximal urinary flow.

b. *Minimal urinary osmolarity* (*Table IIb*): In contrast to the *direct* correlation between total solute excretion and urinary solute concentration at the height of water diuresis, endogenous creatinine clearance *inversely* correlated with the minimal urinary osmolarity; *i.e.*, the higher the creatinine clearance, the more dilute the urine.

This point is not established at a reasonable level of significance when the analysis is based on a single observation per subject but is justified if the 29 experiments are regarded as independent. This point has been referred to above.

c. *Free water clearance* (C_{H_2O}): At the height of water diuresis, C_{H_2O} was *directly* correlated with the magnitude of the creatinine clearance (Table IIc). Inasmuch as C_{H_2O} is a derived, and not an observed value this statistical observation must be accepted with some reservation (6). However, if maximal urinary flow varies directly with cre-

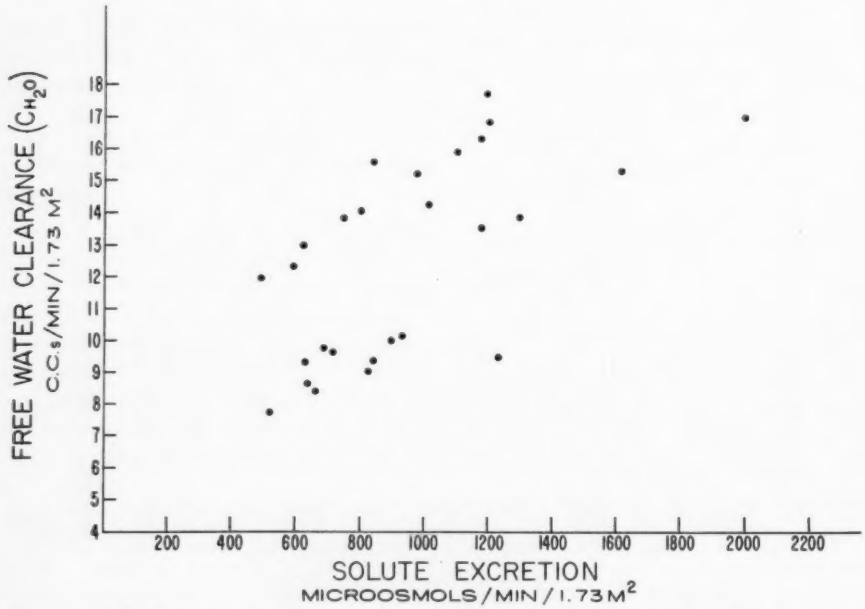

FIG. 4b. THE RELATIONSHIP BETWEEN THE FREE WATER CLEARANCE AND THE RATE OF SOLUTE EXCRETION AT THE HEIGHT OF SUSTAINED WATER DIURESIS

atinine clearance while minimal urinary osmolarity varies inversely, then C_{H_2}, from its derivation, should correlate directly with glomerular filtration rate.

DISCUSSION

If one accepts the concepts that during maximal water diuresis all urinary water is derived from the glomerular filtrate and a minimal amount is reabsorbed in the distal segment of the nephron (7), then the magnitude of the diuresis is dependent on the amount of fluid delivered to this segment. The close correlation between maximal urinary flow and solute excretion (Figure 1) is consistent with this interpretation and substantiates the observations of Rosenbaum, Nelson, Strauss, Davis, and Rossmeisl (8) as well as those of others (9, 10). Although Welt and Nelson (11) concluded that the maintenance of maximal diuresis for long periods of time (4 to 6 hours) was not dependent on the rate of excretion of solute, in only one of their experiments was the maximum diuresis moderately well maintained and in this case the excretion of solute fell very little.

Rosenbaum and his associates (8) gave oral loads of urea and various sodium salts to their sitting subjects during water diuresis and concluded that sodium had a greater effect on urinary flow than the same osmolar quantity of urea. However, the expansion of extracellular volume which occurred with the salt loading, but not with urea, may have produced these differences, possibly by altering glomerular filtration rate or tubular reabsorption of salt and water (12). Statistical evaluation of the data in the present study did not show that tubular water was obligated differently by electrolytes than by urea (non-electrolytes) during water diuresis (Table III).

Observations by Wesson and Anslow (13), and Smith (7) during water diuresis and massive solute loading led these investigators to conclude that, at the height of water diuresis, the free water clearance (C_{H_2O}) was constant for any given animal or individual, and that under these circumstances the rise in urinary flow with increased excretion of solute represented simply the addition of isosmotic proximal tubular fluid to the urine. The close correlation between the rise in the minimal osmolarity and the rise in the excretion of solute (Figure 1) is consistent with this concept. However, in the present investigation, free water clearance was not a constant for any one individual but actually increased with increasing dietary intake and urinary excretion of solute (Figure 4). Similar changes in C_{H_2O} were noted by Rosenbaum and his associates (8) in experiments in which solute excretion was varied during water diuresis, by Welt, Young, Thorup, and Burnett (14) following the administration of a carbonic anhydrase inhibitor to subjects with maximal water diuresis, and in previous experiments from this laboratory (15). This rise in C_{H_2O} can be adequately explained by the reabsorption of additional solute in hypertonic solution, when further isosmotic fluid is delivered to the distal tubule.

The statistical observations in the present study strongly suggest that glomerular filtration rate (creatinine clearance) has an effect on maximal urinary flow, C_{H_2O} and minimal urinary osmolarity, independent of any effect on solute excretion. Ludemann, Raisz, and Wirz (16), studying water diuresis in dogs, observed lower minimal U/P ratios for creatinine and higher maximal rates of urinary flow when creatinine clearance was increased by increasing the protein content of the diet. They thought that the increased excretion of urea could not explain these changes. Unfortunately they did not present data on the effect of the dietary change on total solute excretion. Rosenbaum and associates (8) minimized the role of glomerular filtration in the variations of maximal diuresis, because they demonstrated large changes in solute excretion and urinary flow with minimal changes in glomerular filtration. However, their data did not throw light upon the possible additional importance of changes in glomerular filtration rate.

The mechanisms by which variation in glomerular filtration *per se* could affect maximal urinary flow and C_{H_2O} are not clear. It is possible that a rise in filtration rate would increase the volume of isosmotic fluid delivered to the distal tubule, from which the additional solute but not the water could be reabsorbed. The net effect would be in increased urinary flow without a further increase in the excretion of solute. Shannon, in studies on dogs with diabetes insipidus, suggested another explanation (17). The increased rate of filtration, by increasing the velocity of flow along the proximal tubule, might prevent equilibrium between the actively reabsorbed solute and passively reab-

sorbed water. The net effect would be the delivery of a greater quantity of water but no greater quantity of solute into the distal tubule and a consequent increase in urinary flow.

The statistical observation (Table IIb) that minimal urinary osmolarity varies *inversely* with the magnitude of glomerular filtration is inconsistent with the studies of Leaf, Kerr, Wrong, and Chatillon (18) who concluded from direct experiments in dogs that a decrease in filtration rate had no effect on minimal urinary osmolarity. However, it is supported by the findings of Berliner and Davidson (19), that decreasing the filtration rate in one kidney of a dog at the height of water diuresis causes an increase in the osmolar concentration of the urine above that produced by the contralateral kidney.

The relationships of glomerular filtration and the excretion of solute to the parameters of sustained water diuresis as noted in this study may be of fundamental importance in the pathogenesis of the impaired water excretion of such clinical states as cirrhosis of the liver, congestive heart failure, Addison's disease and panhypopituitarism, in all of which pathological lowering of glomerular filtration rate and/or solute excretion usually occur.

SUMMARY

1. Maximal sustained water diuresis was produced in 29 experiments in 17 normal semirecumbent subjects.

2. The effects of physiological variations in the dietary intake and urinary excretion of solutes and in glomerular filtration rate (endogenous creatinine clearance) upon maximal urinary flow, free water clearance (C_{H_2O}) and minimal urinary osmolarity were evaluated.

3. The magnitudes of the maximal urinary flow, C_{H_2O} and minimal urinary osmolarity were *directly* correlated with the rate of solute excretion.

4. Glomerular filtration rate (endogenous creatinine clearance) *per se* was *directly* correlated with maximal urinary flow and C_{H_2O} and *inversely* correlated with the minimal urinary osmolar concentration achieved during water diuresis.

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THE INTERACTION OF GLUCAGON AND INSULIN ON BLOOD GLUCOSE

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(Submitted for publication December 27, 1955; accepted March 15, 1956)

In previous publications from this laboratory (1, 2) evidence was presented which indicates that glucagon enhances the peripheral utilization of glucose [1] in normal men and dogs as well as in the depancreatized dog. The purpose of this paper is to report experiments on the combined effect of glucagon and insulin on arterial and venous glucose levels in normal men, and to compare the magnitude of this effect with that observed in subjects given similar amounts of each hormone separately.

METHODS

Fifty-six men (ages 21 to 47) considered normal with respect to carbohydrate metabolism and in good general health and nutritional state were studied. Controlled basal conditions were obtained in the following manner: (a) Subjects were hospitalized and placed on a high carbohydrate (360 gm.), high caloric diet (3500 calories) for at least three days before being studied; (b) a 14-hour fast preceded each study; (c) experiments were begun at 8 a.m. following a 30 to 60-minute period of bed rest in the test bed in a special room; (d) venous blood samples were obtained from an indwelling needle inserted (using local Procaine anesthesia) five minutes before the experiment was begun; (e) every effort was made to keep the subjects motionless, relaxed and comfortable during the experiments.

Ten per cent glucose was infused intravenously at a constant rate (250 to 370 mg. per min. in different subjects) throughout the test period by means of a Bowman constant infusion pump. The initial 30 to 60 minutes of the infusion period served as a control period, following which glucagon [2] and/or insulin [2] was added to the infusion bottle. The glucagon dosage was 0.7 to 1.0 mg. administered by constant intravenous infusion over periods ranging from 60 to 100 minutes. Insulin dosage ranged from 0.054 to 0.128 units per min. by constant intravenous infusion for periods ranging from 65 to 110 minutes. Mean insulin dosage for the subjects given insulin alone was .076 units per minute, for the subjects given insulin plus glucagon it was .071 units per minute. The dosage of insulin was arrived at by trial and error; the criteria used were that the dose be sufficient to cause a definite drop in blood sugar, but not so large as to result in hypoglycemic values. Data in which arterial blood sugar fell below 65 mg. per cent were discarded. In the subjects who received both insulin and glucagon, the insulin infusion was begun 15 to 25 minutes before the glucagon in order to obtain the maximum effect of each hormone (on blood sugar level) at approximately the same time.

Venous and capillary (finger) blood specimens were drawn at five or ten-minute intervals, a total of 30 to 40 samples during each experiment. Venous samples were obtained from the antecubital vein which carries mixed blood from the deep and superficial tissue of the forearm and hand (3, 4). Capillary blood contains the same glucose concentration as arterial blood (5). Samples were analyzed for glucose content by the Nelson-Somogyi method (6). Sodium fluoride and immediate refrigeration were used to prevent glycolysis in the samples. Capillary samples were analyzed singly, venous samples in duplicate. In approximately 900 duplicate venous samples the maximum difference between any pair was 10 per cent, and the average was 2 per cent.

The A-V/A data were subjected to a statistical analysis by means of a non-parametric median test (7); *i.e.*, using the medians as a cutting point. The data were placed in a 2×2 contingency table, and then subjected to a chi-square analysis.

RESULTS

The data on the 12 subjects who received insulin following an initial control period are given in Table I. The data from one of these experiments are shown in Figure 1. In 11 of the 12 subjects insulin resulted in a fall in arterial and venous blood glucose levels to or below fasting levels in spite of the constant glucose infusion.

[1] Glucose utilization is used to mean the disappearance of blood glucose.

[2] Generously supplied by Drs. O. K. Behrens and W. R. Kirtley of Eli Lilly Company. The glucagon (Lot 208-158B-214A) contains 50 per cent of the potency of the crystalline standard and an insulin content of 0.05 to 0.005 units per milligram. The insulin (Lot No. T-3206) was a preparation free of glucagon.

TABLE I

Insulin cases *

Patient Wt. (Kg.)	Age	Glucose mg./min.	Insulin units/min.		Time in minutes 0	10	20	30	40	50	60	70	80	90	100	110	120	130
W. Y.	22	344	.128	A†	89	108	108	125	130	134	↑ 136	136	127	118	106	92		
77.8				V†	87	105	105	123	128	132	135	130	121	111	99	85		
E. J.	25	360	.088	A	86	105	114	120	124	127	↑ 129	130	128	117	99	85	77	
79				V	78	100	108	112	115	116	117	117	114	105	93	82	74	
R. B.	29	360	.113	A	97	109	120	129	134	138	↑ 140	135	122	106	90	76		
81.6				V	92	104	115	122	127	131	133	127	116	104	91	78		
C. J.	26	370	.061	A	87	102	116	126	134	140	↑ 144	146	146	141	135	127	122	
70.7				V	82	98	109	117	123	127	130	132	133	133	132	125	117	
R. G.	36	360	.082	A	89	109	120	127	131	133	↑ 134	135	135	122	109	97	87	
69.7				V	88	105	114	121	125	128	130	131	127	113	101	90	81	
J. C.	32	295	.069	A	93	123	138	149	↑ 150	139	118	98	82					
70.5				V	91	113	126	136	138	113	92	80	71					
P. W.	21	270	.073	A	100	117	128	134	↑ 134	129	120	108	97	88	79	72	70	
67				V	95	109	118	123	122	111	94	85	80	75	71	68	66	
J. H.	21	270	.061	A	96	111	122	130	↑ 134	134	132	126	118	108	100	94		
61.3				V	92	105	115	122	125	122	110	101	92	84	76	69		
A. M.	27	250	.054	A	92	100	106	110	↑ 110	109	106	101	97	93	88	83	77	
88				V	83	92	98	102	103	99	94	88	83	78	74	69	65	
R. S.	32	300	.059	A	94	117	133	142	↑ 146	146	146	146	142	136	128	118	108	97
71				V	93	110	124	134	139	139	139	139	136	130	123	112	98	82
W. H.	24	300	.061	A	85	112	128	137	↑ 142	141	137	129	119	108	95	84	76	
58.6				V	82	101	116	126	132	134	126	113	104	88	78	71	63	
K. W.	38	260	.069	A	84	105	113	118	↑ 122	125	124	121	115	108	102	97	92	88
74				V	85	101	108	108	105	98	90	83	77	71	66	61	58	55

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.

† A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).

↑ Beginning of insulin infusion.

A-V/A values [3] with insulin were significantly increased over control values ($P = .01$).

The data on the 12 subjects who received a combined infusion of insulin and glucagon after an initial control period are shown in Table II and one of the experiments is plotted in Figure 2. In eight subjects of this group there was a marked arterial hyperglycemia (over 200 mg. per cent) when glucagon was added to the glucose-insulin infusion mixture; in three there was little or no increase in arterial blood sugar over control values. In 11 of the 12 subjects a marked increase in A-V glucose differences occurred (see Table II). The three subjects who showed little or no arterial hyperglycemia after the addition of glucagon were, nevertheless, among those who exhibited marked increases in A-V glucose differences.

A comparison of the data of the insulin plus glucagon group with the groups who received glucagon (previously reported [2]) or insulin alone revealed the following noteworthy differences: (a) the A-V/A values for the insulin plus glucagon group were significantly greater ($P = .001$) than those for either of the other two groups (Figure 3); (b) the arterial hyperglycemia of the insulin plus glucagon group was often greater than that of the glucagon group despite the fact that the mean glucose infusion rates [4] of the former

[3] A-V/A = arterio-venous glucose difference divided by arterial glucose concentration. This value has been shown to be proportional to the specific rate constant of peripheral glucose utilization under the special conditions of the experiments (2).

[4] The mean glucose infusion rates (mg. per minute) for the three groups were: glucagon 321; insulin 312; and insulin plus glucagon 296.

were slightly lower than those of the latter, and (c) the fall in blood sugar below initial fasting levels so commonly observed in the insulin group did not occur [5] in the insulin plus glucagon group.

DISCUSSION

That insulin enhances the peripheral utilization of glucose is now widely accepted. The recent demonstration (*2*) that glucagon has a similar action which is independent of a secondary release of insulin has already been cited. The experiments reported here indicate that when glucagon and insulin are administered together peripheral glucose utilization is increased beyond that with either hormone alone. In addition, the opposing effect of the two on arterial blood sugar may result, at times, in a virtually unchanged level. The greater arterial hyperglycemia often observed with glucagon plus insulin as compared with glucagon alone may have resulted from higher liver glycogen levels induced by the insulin which was administered 15 to 25 minutes before the glucagon in the subjects who received both hormones.

These findings suggest that insulin and glucagon are capable of functioning jointly to enhance the peripheral utilization of glucose and at the same time, of counteracting the opposing effect of each other on the blood sugar. Judging from the variation in responses observed in different subjects

[5] The only exceptions are the last two venous values on subject T. H.

TABLE II

Insulin + glucagon cases *

Patient Wt. (Kg.)	Age	Glucose mg./min.	Insulin units/min.		Time in minutes 0	10	20	30	40	50	60	70	80	90	100	110	120	130	140
H. C.	33	370	.086	A‡	86	103	114	121	126	130	133 ↑	134	136 ↑↑	158	164	163	151		
71.8				V‡	83	98	109	116	121	124	127 |	127	128 |	141	137	133	129		
D. D.†	39	316	.111	A		130	136	141 ↑	145	138	127 ↑↑	128	140	133	126	119	114		
68.1				V		124	129	133 |	136	126	106 |	105	110	110	105	99	96		
M. R.†	27	333	.065	A		149	150	153 ↑	161	162 ↑↑	175	200	212	209	189	174	165		
66.4				V		132	134	136 |	141	145 |	138	153	159	155	147	137	126		
T. D.	37	265	.062	A	106	128	143	150 ↑	155	157 ↑↑	184	208	220	222	217	208	197	185	172
68.4				V	100	118	132	140 |	145	140 |	142	145	142	138	136	138	145	138	110
J. G.	26	275	.084	A	100	108	121	130 ↑	135	140 ↑↑	163	189	201	197	183	167	157	149	134
53.8				V	95	100	112	121 |	127	132 |	145	171	186	184	171	160	152	144	128
E. H.	37	270	.058	A	93	110	121	126 ↑	129	129 ↑↑	136	163	172	172	159	145	132	122	113
73.4				V	91	104	113	118 |	120	120 |	123	141	143	137	128	119	110	103	100
A. O.	43	320	.064	A	88	100	110	119 ↑	125	135 ↑↑	166	193	213	219	212	205	202	197	176
73.6				V	85	96	105	114 |	119	124 |	136	155	167	174	171	154	148	153	147
C. R.	23	310	.054	A	82	101	115	124 ↑	129	130 ↑↑	161	198	213	201	192	184	177	170	163
71				V	82	101	109	113 |	114	115 |	110	136	140	137	122	116	121	120	116
R. R.	29	290	.062	A	95	113	121	127 ↑	131	135 ↑↑	146	185	199	203	185	170	160	151	137
75				V	92	106	114	120 |	124	128 |	116	163	187	197	181	167	156	147	134
J. C.	34	290	.061	A	90	108	121	129 ↑	133	135 ↑↑	163	189	202	197	190	184	180	177	176
80.5				V	90	101	112	121 |	127	121 |	133	145	150	151	150	149	147	147	151
T. H.	38	250	.065	A	92	111	119	123 ↑	126	128 ↑↑	149	167	166	151	138	126	118	107	91
75.5				V	92	104	112	117 |	119	121 |	122	136	140	123	113	105	97	85	71
J. S.	38	300	.077	A	98	122	141	154 ↑	161	158 ↑↑	179	213	227	230	229	224	212	199	198
79				V	92	113	128	137 |	141	141 |	154	170	174	173	173	180	184	185	183

* Blood sugar values at 10-minute intervals taken from curves constructed from determinations on samples (30 to 40 per subject) drawn at staggered time intervals which varied in the different subjects.

† Control period data not used for statistical analysis because a loading dose of glucose was used.

‡ A—Capillary blood sugar (mg. per cent). V—Antecubital vein sugar (mg. per cent).

↑ Beginning of insulin infusion. ↑↑ Beginning of glucagon infusion.

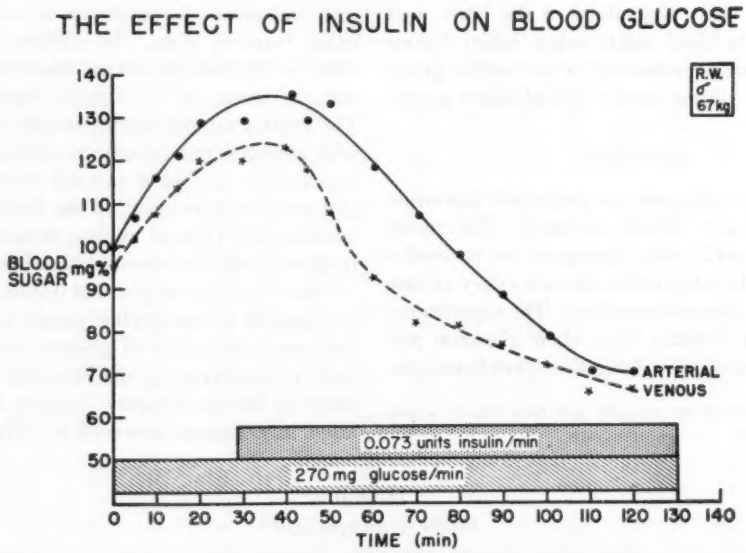

FIG. 1. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

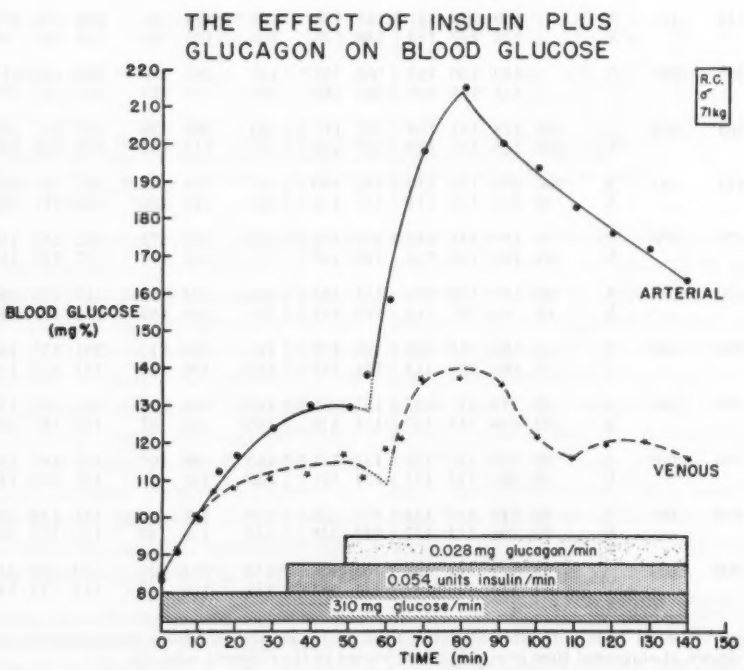

FIG. 2. THE EFFECT OF A CONTINUOUS INTRAVENOUS INFUSION OF GLUCAGON PLUS INSULIN ON ARTERIAL AND VENOUS BLOOD SUGAR LEVELS IN A NORMAL MAN

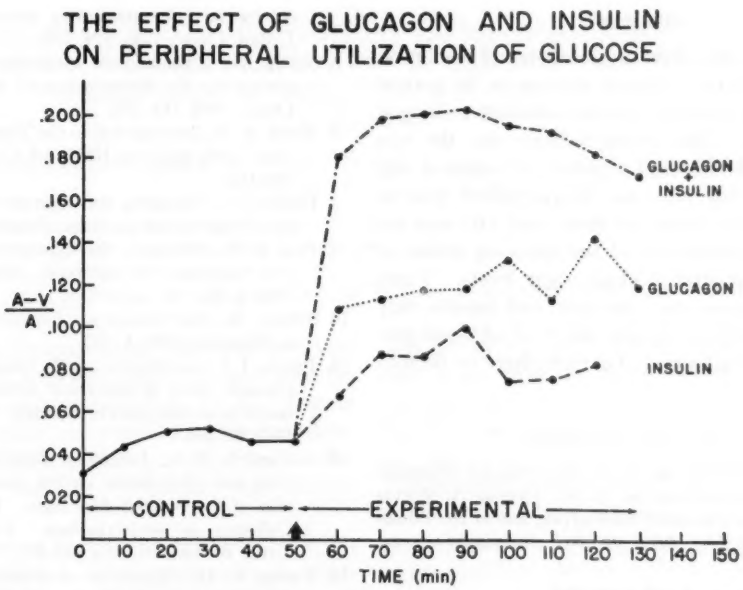

FIG. 3. A SUMMARY OF CONTINUOUS INTRAVENOUS INFUSION EXPERIMENTS IN NORMAL MAN SHOWING THE DIFFERENCE BETWEEN THE EFFECT OF INSULIN, GLUCAGON, AND GLUCAGON PLUS INSULIN ON AN INDEX OF THE PERIPHERAL UTILIZATION OF GLUCOSE (A-V/A)

Each point of the control period represents the median value of 54 subjects, whereas each point of the three experimental groups represents the median value of 12 subjects. The data on the glucagon subjects were taken from a previous publication (2).

receiving similar doses, there is considerable individual difference in responsiveness to the two hormones. No correlation of response with body weight was observed.

The fact that glucagon and insulin have opposing actions on blood glucose and liver glycogen levels has led many to postulate that they are antagonists (8, 9). Another concept of glucagon's role has been advanced by Bürger (10) and by Pincus and Rutman (11). These workers suggest that glucagon acts as an adjunct to insulin because it mobilizes liver glycogen for the enhancing action of insulin on glucose utilization in the peripheral tissues.

It has been reported that glucagon inhibits the action of insulin on glucose uptake by the isolated rat diaphragm (12). This was a preliminary report on work done before purified preparations of glucagon were available. Smith in F. G. Young's laboratory was unable to confirm these results (13). Ingle, Nezamis, and Humphrey observed no effect of glucagon on the disposal of administered glucose in the eviscerated rat (14). Drury, Wick, and Sherill reported that in the eviscerated rabbit glucagon had no effect on glucose oxidation but had a slight inhibiting effect on the disappearance of blood glucose (15). The apparent discrepancy between some of these findings and those reported here might be explained by the studies of Lang, Goldstein, and Levine (16). These workers have presented evidence suggesting that the liver contains a "humoral agent" which enhances peripheral glucose utilization even in the presence of maximal insulin dosage. It is conceivable that glucagon causes the release of this liver factor and that this accounts for the difference between the results in the normal human as compared with the eviscerated animal and the isolated rat diaphragm. In keeping with this hypothesis is the observation that glucagon has no effect on blood sugar levels or arterio-venous glucose differences in the hepatectomized dog (17).

SUMMARY

Studies on the effect of glucagon and insulin on the disappearance of blood glucose in 56 normal men using a constant glucose infusion technique are reported. The results indicate that the two hormones administered together (a) cause a significantly greater increase in peripheral glucose utilization than either one alone, and (b) may result in a neutralization of the opposing action of each other on arterial blood sugar levels. These findings suggest that glucagon and insulin may function jointly to regulate the level of blood glucose and the utilization of carbohydrate by peripheral tissues.

ACKNOWLEDGMENTS

We are indebted to Dr. Lewis Bernstein for assistance with the statistical analysis, to Dr. Thomas A. Witten for constant interest and helpful advice, and to Dr. Cosmo G. Mackenzie for advice on the preparation of the manuscript.

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ALTERATIONS IN BODY COMPOSITION WITH SODIUM LOADING AND POTASSIUM RESTRICTION IN THE RAT: THE TOTAL BODY SODIUM, NITROGEN, MAGNESIUM AND CALCIUM [1]

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(Submitted for publication October 27, 1955; accepted March 15, 1956)

Alterations of muscle composition and acid base balance during potassium deficiency have been the subject of previous investigations (1, 2). During metabolic alkalosis and potassium deficiency there is a loss of cellular potassium and a lesser or disproportionate increase in muscle cell sodium (3–5).

The changes in chloride metabolism have not been clearly defined. During potassium depletion and sodium loading in humans, the presence of edema has been recorded (6) and during experiments concerning potassium deficiency in rats a gain in weight was noted (7). The volume of distribution of bromide in malnourished infants with chronic diarrhea subjected to sodium chloride loading and potassium restriction predicted an increase in total body chloride on a total weight basis (8). On the other hand, balance studies on two patients with chronic diarrhea demonstrated significant deficits of body chloride (9) and data are at hand from rats indicating that muscle chloride content during potassium depletion may be low (2) or high (10). Analysis of the whole rat under these circumstances by one group disclosed no significant alteration of body chloride content (10). From a clinical viewpoint, the changes in body composition during potassium restriction assume importance in the management of therapy.

The present experiments were designed to examine the behavior of total electrolyte and serum electrolyte concentrations when groups of rats were subjected to sodium loading with and without potassium restriction, and in the presence or absence of desoxycorticosterone acetate (DCA). One group of animals was given sodium bicarbonate in the drinking water, two groups received sodium chloride for variable periods, and the fourth group received sodium chloride as well as injections of desoxycorticosterone (DCA). For controls the carcasses of rats on a normal diet and on a normal diet with saline for drinking, with and without DCA, have been analyzed. Because of the possibility that some of the changes observed were due to a diminished food intake, a study of the changes in carcass composition during starvation has also been included.

The results indicate that the severity of potassium deficiency, as measured by total carcass potassium content, cannot be accurately predicted by any specific change in body composition or by alteration in acid base balance. Total body chloride is usually decreased but, under certain conditions, total chloride may be increased. The status of total body chloride does not correlate with the serum chloride concentration. While total body sodium increased in all potassium deficient animals, the increment could not be correlated with the decrement in body potassium.

EXPERIMENTAL PROCEDURE AND METHODS

Male rats derived from the Wistar strain were used. Normal values for total body electrolyte, water and nitrogen, furnishing standards for comparison of the results on the experimental animals, were determined by carcass analysis of animals of normal health and vigor of varying ages and weights. The larger and older animals had been previously used for breeding purposes. All had been under observation for a period of at least two weeks prior to sacrifice and for one week had been maintained on a synthetic diet of composition previously described (11).

The 48 animals selected for the experimental groups were in most cases of the median weight of the normal animals, 210 to 250 grams. In some instances animals of

[1] This investigation was supported in part by a research grant (H-1638) from the National Heart Institute of the National Institutes of Health, United States Public Health Service.

greater weight were included. They were placed either on a normal or a potassium deficient diet. The normal diet is that described previously (11). The potassium deficient diet was similar to the normal in composition except that potassium was removed and a mixture of sodium chloride and sodium bicarbonate added. A kilogram of potassium deficient diet contained 300 mEq. of sodium, 150 mEq. each of chloride and bicarbonate and 0.5 mEq. of potassium. The animals were divided into 6 groups and subjected to the following conditions:

Group 0. Ten rats received a normal diet and isotonic sodium chloride for drinking during a 10-day period.

Group 1. Thirteen rats received the low potassium diet for three weeks and isotonic sodium bicarbonate for drinking during the last four days prior to sacrifice.

Group 2. Five rats received the low potassium diet for three weeks and isotonic sodium chloride for drinking during the last four days.

Group 3. Five rats received the same treatment as Group 2 but in addition injections of desoxycorticosterone acetate in oil, 2½ mg. daily, were given intramuscularly during the last four days.

Group 4. Four rats were subjected to the same treatment as those in Group 3 except that they were given a normal diet.

Group 5. Six rats received the low potassium diet for eight days and isotonic sodium chloride to drink for eight days prior to sacrifice.

Experiments were also done on a group of five rats to determine the effect of starvation on body composition. These animals were placed in individual cages and given 85 ml. of water daily in which were dissolved 1.7 mEq. of sodium chloride, 4.2 gm. of glucose and a mixture of vitamins. The daily allowance of fluid was completely consumed each day. After eight days of starvation, the animals were sacrificed.

The technique for reducing the rat carcass to a fine, homogeneous, fat free powder and the methods for estimation of carcass chloride, potassium and water and for serum chloride have been described previously (11). The serum CO_2 content was determined by the method of Van Slyke and Neill (12) on blood collected anaerobically from the aorta. Serum sodium and potassium levels were determined by flame photometry.

For total carcass sodium estimation the method of Butler and Tuthill was used (13). One gram of fat free dry carcass was weighed out in a platinum crucible, covered with a nickel lid, and placed in a muffle furnace at 550°C for 20 hours. After cooling the ash was dissolved in 1.5 ml. of 2 N HCl. One drop of alcoholic phenophthalein solution was added and the mixture transferred to a 5-ml. volumetric flask and made up to volume. The solution was then poured into a 10-ml. centrifuge tube and 200 mgm. of Ca $(OH)_2$ were added. The procedure of Butler and Tuthill was then completed. The estimation of total nitrogen was carried out by the method of Conway (14) using 50 mgm. of fat free dry carcass for digestion in a 50-ml. Kjeldahl flask. Each digestion was done for at least eight hours in duplicate and microdiffusion technique was carried out in triplicate. Blanks and a standard solution were run simultaneously. For digestion 3.5 ml. of Conway's acid digestion mixture were employed. For magnesium and calcium determinations 500 mgm. of carcass were ashed and the ash dissolved in weak HCl. To a 2.0-ml. aliquot of the extract of the ash was added 2.0 ml. of saturated ammonium oxalate and the calcium precipitated. To 2.0 ml. of the supernatant fluid were added 2 ml. of 2 per cent KH_2PO_4 and 2 ml. of concentrated ammonia to precipitate magnesium as the triple salt—ammonium magnesium phosphate. After repeated washing and centrifuging the triple salt was dissolved in 0.2 N HCl and the method of Fiske and Subbarow (15) was then employed to determine the amount of phosphate present. The content of magnesium was calculated from a standard curve for the triple salt derived from estimations of standard magnesium solutions. Calcium was estimated by the permanganate titration method, after the calcium oxalate precipitate had been repeatedly washed and dissolved in 2.5 ml. of 30 per cent sulphuric acid.

CALCULATIONS

The chloride space has been used for appraisal of change of extracellular volume and no attempt has been made in the calculation to correct for intracellular chloride or for the slightly greater chloride concentration in connective tissue water as compared with that of a serum ultrafiltrate. It is assumed that neglect of these corrections will produce the same magnitude of error in the experimental as in the normal rats so that the values obtained provide an index of change in extracellular volume. A value of 0.95 is used as the Donnan factor and it is assumed that serum water represents 930 ml. per liter of serum.

The total sodium of the carcass has been partitioned into two fractions, that present in the chloride space and that deposited in the bone and cells. The calculation is based on the assumption that the concentration of sodium throughout the chloride space is the same as that in an ultrafiltrate of serum. That which is not in the chloride space serves as an index of the amount of intracellular and bone sodium. The actual amount in bone and cells is probably greater than that calculated, because the chloride space has not been corrected for factors mentioned above, and is consequently larger than it should be. Hence, it should be emphasized that the calculated values for sodium in the non-chloride space serve only as an index of bone and cell sodium.

RESULTS

Body composition of normal rats

In normal rats total body electrolyte (sodium, potassium, chloride, calcium and magnesium), nitrogen and water were found to be linearly related to the fat free dry solid content of the car-

cass with relatively small scatter. The regression equations expressing the relation of these parameters to fat free dry solid together with the standard errors of estimate are given in Table I. Also included in the table is the regression for bone and cell sodium on fat free dry solid and the average and range for body fat content.

The rats sacrificed to establish the normal values for body electrolyte, water and nitrogen ranged in body weight from 70 to 410 grams, and in fat free dry solid weight from 13 to 96 grams.

TABLE I

Body composition in the normal rat: Regressions of total body electrolyte, nitrogen and water on fat free dry solid *

Regression equations			Standard error of estimate	Number of rats
Na_t (mEq.)	$=0.193$ FFDS$+$	0.78	$\pm$ 0.64	36
Na_{b+c} (mEq.)	$=0.038$ FFDS$-$	0.38	$\pm$ 0.55	28
K_t (mEq.)	$=0.283$ FFDS$+$	0.36	$\pm$ 1.08	47
Cl_t (mEq.)	$=0.131$ FFDS$+$	1.04	$\pm$ 0.41	35
Mg_t (mEq.)	$=0.101$ FFDS$+$	0.59	$\pm$ 0.32	32
Ca_t (mEq.)	$=2.20$ FFDS$-$	1.74	$\pm$11.50	35
N_t (gms.)	$=0.131$ FFDS$+$	0.07	$\pm$ 0.20	40
H_2O_t (ml.)	$=2.725$ FFDS$+$	13.2	$\pm$ 3.70	36
Average fat content: 9.6% body wt. Range 15.3 to 3.3%				36

* Subscript "t" indicates amount in total carcass; Subscript "b+c" indicates amount in bones plus cells; FFDS = fat free dry solid.

Significance of weight changes with potassium deficiency

Animals used in the experiments to determine the effect of potassium deficiency weighed 210 to 250 grams initially. In some of the groups larger animals were included. The range of fat free dry solid weights at sacrifice extended from 33 to 70 grams. In all groups except Group 5 the experimental conditions were such that weight changes during the experiments were relatively slight. While on the potassium deficient diet the animals initially gained weight. Subsequently when electrolyte solution replaced tap water for drinking, some of the groups showed a slight loss of weight. Thus the animals of Groups 0, 1, 2 and 3 underwent a slight weight loss while those of Group 4 receiving a normal diet, DCA and saline to drink showed a small weight gain. In all cases the changes in weight amounted to less than 8 per cent of the initial weight. It seems likely that in these groups the weight changes were mainly the result of changes in body fat and water content with little loss of tissue (measured as fat free dry solid). Evidence of water loss was seen on carcass analysis in Groups 0, 1 and 2. Body water in these animals (as compared with the normal animal of the same fat free dry solid content) was reduced by 4.6, 5.8 and 2.6 per cent, respectively (Figure 5 and Table II). Presumably there were also small changes in body fat content. As compared with an average fat content of 9.6 per cent of body weight in the normal animals, the fat content of Group 0 animals averaged 8.6 per cent, the Group 1 animals, 9.2 per cent, Group 2, 7.7 per cent and Group 3, 9.5 per cent. In the Group 4 animals, which gained weight during the experimental procedure, there was a gain in water of 2.2 per cent (Table II) and fat content at sacrifice averaged 12.5 per cent of body weight. The data would indicate that the experimental conditions to which the animals of Groups 0 to 4 were subjected produced little change in their nutritional status. Weight changes appeared to result primarily from small changes in body water and fat. It would appear that body composition in these groups can be adequately assessed by comparing it with that of a normal rat of the same fat free dry solid content.

In contrast, the experimental conditions to which the animals of Group 5 were subjected were more rigorous. In the course of the experiment a weight loss averaging 16.7 per cent of the initial weight occurred. A fat content at sacrifice averaging 5 per cent of body weight suggested that their caloric intake had been low. The fat loss, however, is not of sufficient magnitude to account for their total weight loss and losses of fat free dry solid together with commensurate amounts of water would be predicted. Reduction of fat free dry solid complicates the assessment of the data of this group. Therefore, an attempt at assessment of the body composition changes occurring in these animals has been made not only by comparing body composition at sacrifice with that of a normal animal of the same fat free dry solid content but also by comparing composition with that calculated to exist at the outset of the experiment. Further assessment is provided by comparison with the data for animals subjected to simple starvation.

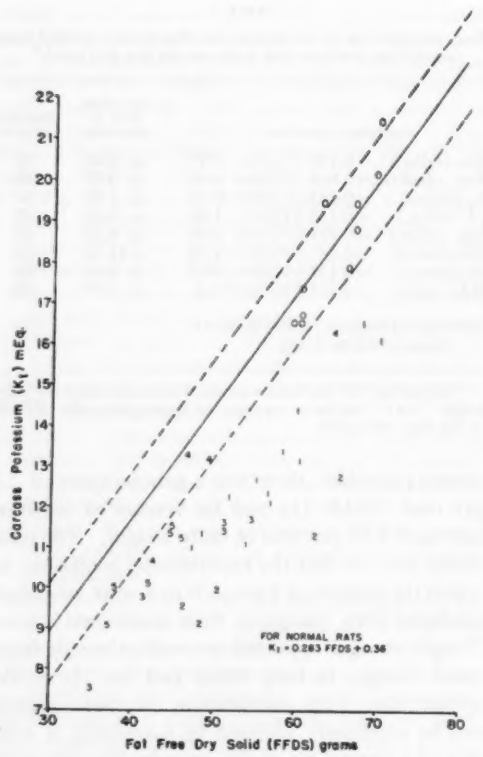

FIG. 1. CARCASS POTASSIUM OF THE RAT VERSUS CARCASS CONTENT OF FAT FREE DRY SOLID

The points for the experimental animals are designated by numbers corresponding to the experimental group. The straight line represents the regression of carcass potassium on fat free dry solid for 47 normal animals fed a normal diet and the broken lines, the standard error of the estimate.

Effect of potassium deficiency, sodium loading and DCA on body composition

In Figure 1 total carcass potassium content of the experimental animals has been plotted against their fat free dry solid content at sacrifice. The values have been indicated by numbers corresponding to the group to which the animal belongs. The potassium content of normal animals is indicated by the line bounded by the broken lines (standard error of estimate). In comparison with a normal animal of the same fat free dry solid content it is evident that all animals on a potassium restricted diet drinking either saline (Groups 2, 3 and 5) or sodium bicarbonate (Group 1) were potassium depleted. Four animals receiving a normal diet, saline to drink and injection of DCA (Group 4) fall in or close to the normal range in potassium content. Animals receiving a normal diet and saline to drink over a ten-day period (Group 0) demonstrated no deficit of carcass potassium.

Carcass chloride and sodium content are shown in similar plots in Figures 2 and 3. Again, the average and standard error of the estimate for normal animals is indicated by the line bounded by the broken lines. According to this method of assessment, body composition with respect to chloride showed considerable variation with the various regimens employed. Animals receiving a potassium restricted diet and additional sodium loads for drinking for a four-day period, with or without DCA (Groups 1, 2 and 3), had a low total chloride while the animals of Group 5 receiving eight days of saline with only eight days of potassium restriction had a high total chloride per

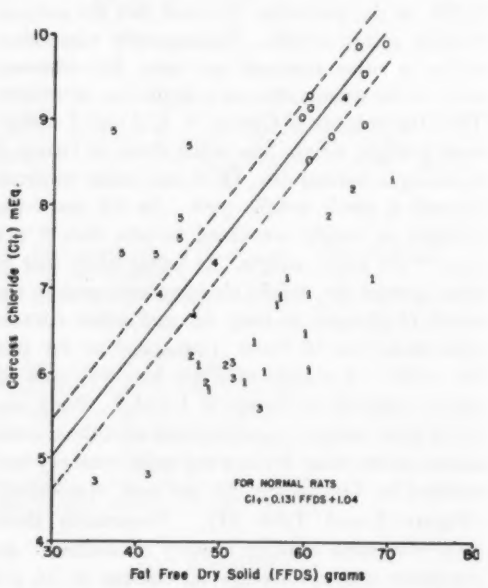

FIG. 2. CARCASS CHLORIDE OF THE RAT VERSUS CARCASS CONTENT OF FAT FREE DRY SOLID

The regression of carcass chloride on fat free dry solid for 35 normal animals fed an adequate diet is indicated by the straight line and the standard error of the estimate, by the broken lines.

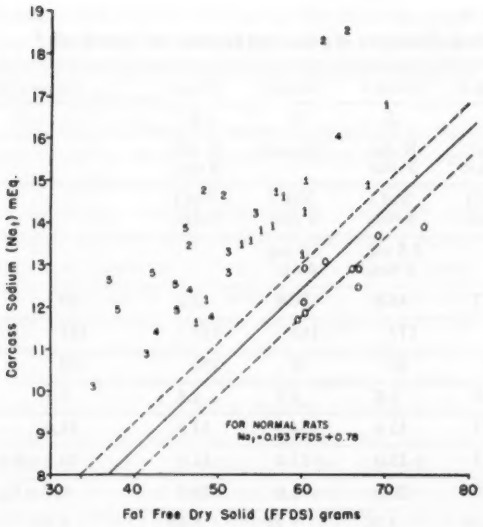

FIG. 3. CARCASS SODIUM VERSUS CARCASS CONTENT OF FAT FREE DRY SOLID

The straight line represents the regression of carcass sodium on fat free dry solid for 36 normal animals on a normal diet and the broken lines, the standard error of the estimate.

unit of fat free dry solid. As will be indicated later the high carcass chloride in this group is probably secondary to the poor nutritional state of the animals rather than the effect of potassium depletion. Both groups on a normal diet (Groups 0 and 4) had a normal total chloride.

The changes in body sodium content, as may be seen in Figure 3, were, on the other hand, more constant. An increased body sodium content was present in all animals receiving a potassium restricted diet (Groups 1, 2, 3, and 5), as well as in those receiving a normal diet, saline and DCA (Group 4). A normal or slightly low body sodium content was seen only in animals on a normal diet with saline for drinking (Group 0).

Values serving as an index of sodium in bone and cells are shown in Figure 4. By the method of calculation employed, the average bone and cell sodium for normal rats, indicated by the solid line, is about 12 per cent of the total in smaller animals and about 17 per cent in larger animals. The rats of Groups 1 and 2 receiving a potassium deficient diet and sodium bicarbonate or saline for drinking had a marked increase in the amount of sodium deposited outside of the chloride space. In the remaining groups the amount of sodium in cells and/or in bone was normal or only slightly altered.

None of the experimental animals showed a deviation from the normal in the relation of total body nitrogen, magnesium, or calcium to fat free dry solid. The graphs expressing these relationships are not shown.

Carcass water content is shown in Figure 5. Total water was definitely altered in the animals receiving a normal diet and saline to drink (Group 0) and in those subjected to a potassium deficient diet and sodium bicarbonate for drinking (Group 1). In these groups body water tended

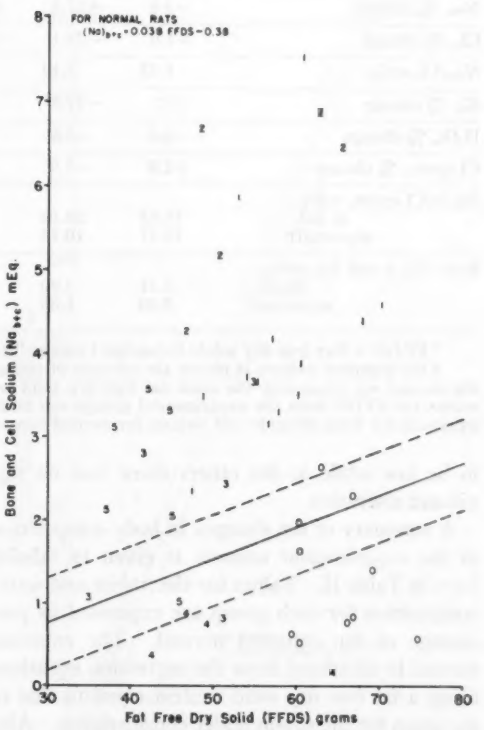

FIG. 4. THE BONE AND CELL SODIUM OF THE RAT (CALCULATED AS THE AMOUNT DEPOSITED OUTSIDE THE CHLORIDE SPACE) VERSUS THE CARCASS CONTENT OF FAT FREE DRY SOLID

The straight line represents the regression of bone and cell sodium on fat free dry solid for 28 normal rats on a normal diet and the broken lines, the standard error of the estimate.

TABLE II

Determined and derived values for body composition and serum electrolytes in potassium depleted and control rats *

	Group 0	Group 1	Group 2	Group 3	Group 4	Group 5	Normal rats
Number of rats	10	13	5	5	4	6	
Diet	Normal	K def. 3 wks.	K def. 3 wks.	K def. 3 wks.	Normal	K def. 8 das.	
Drinking fluid	NaCl 10 das.	$NaHCO_3$ 4 das.	NaCl 4 das.	NaCl 4 das.	NaCl 4 das.	NaCl 8 das.	
DCA				2.5 mg. 4 das.	2.5 mg. 4 das.		
FFDS, gms./rat	64.7	57.6	54.7	46.8	50.8	42.2	50
Serum Na, mEq./L.	145	153	151	172	165	143	145
Serum Cl, mEq./L.	107	90	93	84	94	102	110
Serum K, mEq./L.	4.6	2.0	1.8	1.8	2.3	2.5	4.7
Serum CO_2 content, mEq./L.	24.0	38.5	36.2	45.6	34.3	33.6	24.0
Na_t, % change	−3.8	+17.5	+40.7	+25.0	+23.4	+41.9	10.4 mEq.
Cl_t, % change	−1.0	−23.1	−15.0	−24.0	−2.0	+20.0	6.7 mEq.
Na_t/Cl_t ratio	1.35	2.12	2.32	2.26	1.71	1.62	1.37
K_t, % change	+1.1	−27.5	−33.2	−23.1	−9.1	−14.0	14.5 mEq.
H_2O_t, % change	−4.6	−5.8	−2.6	+0.3	+2.2	+3.7	149.4 ml.
Cl space, % change	+1.8	−5.8	+0.3	+0.2	+15.0	+31.5	60.8 ml.
Na in Cl space, mEq.							
found	11.45	10.10	9.86	10.00	11.82	10.10	
expected†	11.17	10.13	9.62	8.44	9.05	7.70	8.90
Bone Na + cell Na, mEq.							
found	1.31	3.90	6.08	2.30	1.10	2.54	
expected†	2.10	1.80	1.71	1.38	1.53	1.22	1.50

* FFDS = Fat free dry solid; Subscript t means "total"; DCA = Desoxycorticosterone acetate.

† By expected sodium is meant the amount of sodium either inside or outside the chloride space that is present in the normal rat possessing the same fat free dry solid as that of the experimental group under consideration. Mean values for FFDS from the experimental groups are taken for comparison. The expected sodium is derived from the equations for total chloride and sodium for normal rats from 70 to 410 grams in weight.

to be low while in the others there was no significant alteration.

A summary of the changes in body composition in the experimental animals is given in tabular form in Table II. Values for electrolyte and water composition for each group are expressed as percentage of the expected normal. The expected normal is calculated from the regression equations using a fat free dry solid content equal to that of the mean for the group under consideration. Also shown in the table are the serum contents of sodium, chloride, potassium and carbon dioxide as well as derived values as to the amount of sodium in the chloride space and in bone and cells.

The rats receiving a normal diet and saline for drinking over a ten-day period (Group 0) demonstrated no gross change in body composition except for a slight reduction in total body water. There was no change in the $\frac{\text{total Na}}{\text{total Cl}}$ ratio or in the volume of the chloride space.

In rats of Group 1 subjected to potassium restriction for three weeks with four days of sodium bicarbonate for drinking, body potassium was reduced by 27 per cent. Also there was a moderately severe hypochloremic alkalosis with a slight elevation of serum sodium concentration. The total carcass sodium was elevated and total chloride reduced, resulting in a ratio of total chloride to total sodium of 2.12 as compared with a normal value of 1.37. There was a significant reduction of total water as well as in the volume of the chloride space; a small water loss from both extra- and intracellular compartments would be pre-

dicted. The sodium content of the chloride space was calculated as unchanged so that the increment in total sodium apparently resided entirely in cells and/or bone.

The rats of Group 2 were subjected to a regimen similar to that of Group 1 except that sodium chloride rather than sodium bicarbonate was given for drinking. Body composition resembled the Group 1 animals with respect to the loss of potassium and chloride and the degree of alkalosis. The increment in total body sodium was, on the other hand, much greater, and again, nearly all the excess was located in cells and/or in bone.

When DCA was added to the regimen of potassium restriction and saline loading (Group 3) the severity of the potassium depletion was not enhanced. Indeed the potassium loss was less appreciable than that of animals receiving the same regimen without DCA (Group 2). Alkalosis was marked with a very high serum sodium and a low serum chloride concentration. While the gain in body sodium and loss of body chloride was similar to that of the previous groups (Groups 1 and 2), the increment of sodium remained largely in the chloride space with only a small gain in cell or bone sodium.

Animals receiving a normal diet with saline and DCA (Group 4) also showed considerable change in body composition. Carcass potassium content tended to be low in two of the four animals, and alkalosis was present. The origin of the alkalosis appeared to differ from that of potassium restricted animals in that it was mainly the result of sodium retention in the extracellular phase with little change in body chloride content. The average value for bone and cell sodium was, if anything, somewhat reduced. The serum sodium concentration was elevated and serum chloride low; the latter apparently resulted from the expansion of the chloride space in the face of a normal body chloride content.

It is of interest to compare the changes in composition of the rats in Group 3 receiving the combination of potassium restriction and DCA with those in Group 2 receiving only potassium restriction and those in Group 4 receiving only DCA (all 3 groups received saline for drinking). DCA alone (Group 4) appeared to cause a slight loss in body potassium but when given with potassium restriction (Group 3) there was no augmentation of potassium loss. DCA alone caused accumulation of sodium in the chloride space while potassium deficiency resulted in a deposition of sodium outside the chloride space. When the two are given together as in the Group 3 rats, both extracellular and intracellular and/or bone sodium are increased. DCA alone produced no change in body chloride content (Group 4), and potassium deficiency alone caused a moderate reduction. When the two were given together (Group 3), there was a marked reduction in body

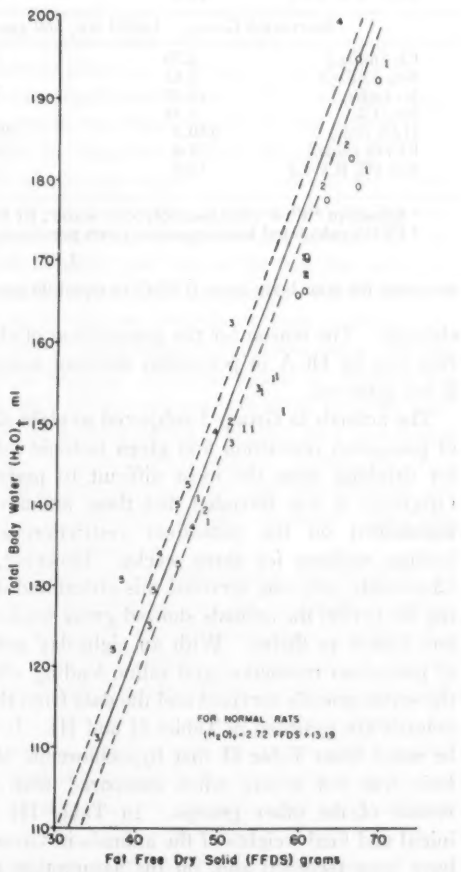

FIG. 5. TOTAL BODY WATER OF THE RAT PLOTTED AGAINST THE CARCASS CONTENT OF FAT FREE DRY SOLID

The straight line represents the regression of body water on fat free dry solid for 36 normal animals on a normal diet.

TABLE III

Calculated initial and final body composition of animals in Group 5 and of animals subjected to starvation—average values *

Body component	Calculated initial composition† A	Composition at sacrifice: Found B	Composition at sacrifice: Change from initial $\frac{B-A}{A} \times 100$	Composition of normal animal with FFDS content = Exper. group: Predicted C	Composition of normal animal with FFDS content = Exper. group: Deviation of exper. group $\frac{B-C}{C} \times 100$
Group 5. Initial wt., 215 gms. Final wt., 179 gms. Wt. loss, 16.7%. N = 6					
Cl_t (mEq.)	7.74	7.82	+ 1.0%	6.58	+20.0%
Na_t (mEq.)	10.66	12.64	+18.6%	8.93	+41.9%
K_t (mEq.)	14.86	10.58	−28.8%	12.31	−14.0%
Na_t/Cl_t	1.38	1.62		1.36	
H_2O_t (ml.)	152.7	133.0	−12.9%	128.2	+ 3.7%
FFDS (gms.)	51.2	42.2	−17.6%	42.2	
Fat (% B.Wt.)	10.0	5.0			
Starvation Group. Initial wt., 160 gms. Final wt., 121.5 gms. Wt. loss, 24%. N = 5					
Cl_t (mEq.)	5.70	5.40	− 5.3%	4.77	+13.2%
Na_t (mEq.)	7.65	7.11	− 7.1%	6.27	+13.4%
K_t (mEq.)	10.42	7.54	−27.7%	8.42	−10.5%
Na_t/Cl_t	1.34	1.32		1.31	
H_2O_t (ml.)	110.2	90.5	−17.7%	90.8	− 0.3%
FFDS (gms.)	35.6	28.5	−20.0%	28.5	
Fat (% B.Wt.)	10.0	2.1			

* Subscript "t" = total electrolyte or water; FFDS = fat free dry solid; N = number of rats.
† FFDS calculated from equation given previously (11),

$$LBM = 3.69\ FFDS + 12.61$$

assuming the lean body mass (LBM) to equal 90 per cent of body weight (body fat = 10 per cent of body weight)

chloride. The reason for the potentiation of chloride loss by DCA in potassium deficient animals is not apparent.

The animals in Group 5 subjected to eight days of potassium restriction and given isotonic saline for drinking were the most difficult to prepare. Originally it was intended that these animals be maintained on the potassium restriction-saline loading regimen for three weeks. However, of 13 animals, only one survived this ordeal and during the period the animals showed gross weakness and failure to thrive. With an eight-day period of potassium restriction and saline loading six of the seven animals survived and the data from these animals are recorded in Tables II and III. It can be noted from Table II that hypochloremic alkalosis was not severe when compared with the results of the other groups. In Table III the initial and final weights of the animals in Group 5 have been recorded and, on the assumption that 10 per cent of their initial weight is fat, the total electrolyte, water and fat free dry solid present at the outset of the experiment has been calculated (column A). The same parameters have been recorded for the actual analysis of the carcass at the time of sacrifice (column B). In column C are given the values for total electrolyte and water which one would expect to find in a normal rat of the same fat free dry solid content as the experimental animals at sacrifice. In the lower half of the table the same procedure has been followed to record data obtained from rats subjected to starvation for an eight-day period. Percentage differences between the predicted and estimated values are shown for convenience.

In both the Group 5 animals and in those subjected to starvation, fat free dry solid and total water were lost with progress of time. The loss of water was commensurate with the loss of fat free dry solid so that the ratio of total water to lean body mass remained essentially the same as that described by Pace and Rathbun for the normal animal (16). In both groups the fat free dry solid at the time of sacrifice is of different composition from that of a normal animal of the same fat free dry solid content. In the starved group, potassium per unit of fat free dry solid is low and sodium and chloride increased. An increase in extracellular water per unit of total water would be predicted. With respect to the elec-

trolyte content of the animals at the outset of the experiment, starvation produced a loss of potassium and only a slight fall in sodium and chloride. The Na_t/Cl_t ratio is maintained at the normal level. In Group 5 the changes in carcass composition resemble those produced by starvation, but the increase of carcass sodium is disproportionately greater than the increase of carcass chloride. Since the main difference between these two groups is the difference in sodium intake, it would seem reasonable to ascribe the disproportionately high carcass sodium of Group 5 and the alkalosis to sodium loading with an inability to excrete sodium effectively.

DISCUSSION

The imposition of various deleterious situations on the homeostatic mechanisms that regulate electrolyte balance appears to produce a wide spectrum of complex changes in body composition that cannot be fully explained. The present investigations pertaining to sodium loading and potassium deficiency emphasize this complexity and reveal the variety of changes in body composition that are possible with metabolic alkalosis. Determination of the electrolyte content of individual tissues may not portray the alterations in total body electrolyte. Analyses of the muscle of potassium deficient animals have suggested that total body chloride is commonly normal (*7*), whereas in the present study it was usually found to be diminished. It would appear that carcass composition can be considerably influenced by the method used to induce potassium deficiency. The administration of DCA, inadequate caloric intake and the kind of sodium salt administered with the potassium restriction regimen all appear to superimpose alterations in the basic changes in body composition and acid base balance produced by potassium deficiency. It would appear that a consistent program must be followed if results are to be critically compared.

The study would indicate that adequate potassium intake is essential to the preservation of homeostasis. Administration of large loads of saline as drinking water to animals in which potassium intake was adequate (Group 0) caused no change in body composition, while administration of saline to potassium restricted animals produced marked changes in body composition. While the experiments represent extreme conditions, the results offer a warning from a clinical standpoint against the unrestricted use of saline during periods of diminished potassium intake.

The consistent changes in body composition which occurred in all groups of potassium restricted, sodium loaded animals were a diminution in carcass potassium and an increase in carcass sodium. The changes in carcass chloride were not so consistent. Chloride was diminished in all groups in which body weight remained relatively constant during the experiments, while animals which showed a marked weight loss in the course of the experiments (Group 5) had an increase in carcass chloride. The data suggest that starvation may significantly affect body composition and changes in nutritional status of the animal must always be considered when interpreting the results of carcass analysis. In the starved animal with progress of time, there appears to be little change in the body content of sodium and chloride but at sacrifice, per unit of fat free dry solid, sodium and chloride are increased. The animals of Group 5 differed little in carcass chloride content from those subjected to starvation without sodium loading. It seems logical to attribute the high chloride content per unit of fat free dry solid in the animals of Group 5 to their poor nutritional status. With respect to carcass sodium, the Na_t/Cl_t ratio and the status of acid base balance, the Group 5 animals differed markedly from the starvation group.

The observations with respect to chloride in the present study suggest that the correction of potassium deficits by the administration of potassium alone without chloride, as described by Cooke, Segar, Reed, Etzwiler, Vita, Brusilow, and Darrow (*7*) would not under all circumstances be successful. The frequent occurrence of a low total body chloride would indicate that for complete repair of body deficits, chloride must be given with potassium.

Although the potassium restricted groups were comparable in the direction of change in sodium and, with the exception of Group 5, in chloride, they were not comparable in many other respects. There was no correlation, for example, between the extent of potassium loss and of sodium gain in

the various groups. It is now well recognized that in the potassium deficient animal, part of the increment of the total body sodium is located in muscle cells and the replacement of sodium for potassium in these cells has been set at a ratio of approximately two sodium ions for three potassium ions (3, 17). It is apparent, however, from the present study that in the whole rat there is no consistent correlation between potassium loss and sodium gain. In the rats of Group 1 potassium loss averaged 4.58 mEq. and the sodium gain was less than half this amount, 2.08 mEq. In Group 2 the sodium gain (4.62 mEq.) was nearly as great as the potassium loss (5.25 mEq.) and in Group 5 the sodium increment of 4.75 mEq. greatly exceeded the potassium loss of 1.72 mEq. These discrepancies do not, of course, contradict the observation that in the muscle cell, three potassium ions are lost for every two sodium gained. Changes in extracellular volume, and perhaps deposition of sodium in bone, appear to cause the variations in this ratio in the whole animal. Use of the calculated values for sodium outside the chloride space (bone and cell sodium) to determine the cellular sodium : potassium ratio is dubious in view of the approximate nature of the calculations and because of the fact that some of the increment in non-extracellular sodium may be in bone.

The inconsistent changes in sodium and potassium balance in the whole animal were paralleled by a similar lack of uniformity in total cation balance. In agreement with earlier muscle analyses (18), the body content of magnesium and calcium was unaltered in potassium deficiency so that changes in the fixed cation in the body were solely dependent on changes in sodium and potassium. In the various groups the fixed cation balance varied from − 3.2 to + 2.3 mEq. per 50 gm. of fat free dry solid.

A lack of correlation is also apparent between the changes in acid base balance and the severity of the potassium deficiency. The increment in serum bicarbonate concentration was approximately the same in groups with potassium losses varying from 9 to 33 per cent. In potassium restricted animals receiving DCA, potassium loss was not enhanced, yet there was a marked elevation in serum bicarbonate. The serum level of potassium was roughly correlated with the severity of the potassium deficit but the total range of variation in the deficient groups was only 0.7 mEq. per L. The carcass content of sodium and chloride was in no way reflected in the serum levels of these ions.

A number of factors appears to influence the pattern of body composition in potassium deficient animals. As mentioned above, starvation seems to be a factor of great importance. Likewise the kind of sodium salt administered appears to have some effect on composition. Animals receiving supplementary sodium bicarbonate for four days with potassium restriction (Group 1) had a much smaller gain in total body sodium than those on the same regimen but receiving sodium chloride (Group 2) although the severity of potassium depletion in the two groups was approximately the same. DCA could be considered a third factor altering the results of carcass analysis. Animals restricted in potassium intake and receiving this steroid had a marked increase in the sodium content of the extracellular phase (Group 3) as compared with animals on a similar regimen but not receiving DCA (Group 2). Because the extracellular volume of the animals receiving DCA was not expanded, a severe hypernatremia resulted which in turn potentiated the hypochloremic alkalosis of potassium deficiency. The results indicate that this steroid not only superimposes further alterations in body composition but also is of little value in facilitating the production of potassium deficiency in the experimental animal. In fact animals on a potassium deficient diet receiving DCA had a slightly less severe potassium depletion than the comparable group not receiving DCA.

The effects of DCA in animals on a normal diet (Group 4), as seen in the present study are in complete agreement with the observations of others. Hypernatremia (19–21) with an increase in exchangeable sodium (21, 22) is also seen following the administration of DCA to dogs. Exchangeable chloride remains normal (21). Muscle potassium (20) as well as total body potassium (22) is decreased and serum potassium levels are low. Similar to the present study, this steroid may also produce an alkalosis in dogs which appears to be independent of potassium loss.

Gamble (21) found that the alkalosis would disappear when DCA was withdrawn without potassium being present in the diet. The average potassium loss in the Group 4 animals in the present study approximated the amount that has been termed labile potassium—an amount that can be lost without alteration in acid base balance.

SUMMARY

1. To ascertain the changes in body composition produced by potassium restriction, groups of normal rats were given diets low in potassium and either isotonic sodium chloride or sodium bicarbonate for drinking for variable periods of time. DCA was administered to some of the groups. The control groups consisted of animals on a normal diet with isotonic saline for drinking with and without DCA and of animals subjected to starvation. The results were assessed by comparing the body composition of the experimental animals with that of normal animals.

2. The basic change in the composition of the potassium restricted-sodium loaded animals was an increase in total body sodium, a reduction in potassium, and, in those animals remaining in good nutrition, a fall in body chloride. Animals losing weight in the course of the experiments, and control animals subjected to starvation without sodium loading, had a definite increase in chloride per unit of fat free dry solid but little change in body chloride content as compared with the content prior to experimentation. Starvation appears to alter markedly body composition with respect to chloride, sodium and potassium.

3. Animals given saline to drink and a diet adequate in potassium had no changes in body composition. With sodium loading, the administration of potassium is necessary for preservation of homeostasis.

4. The potassium deficient animals showed no deviation from the normal in their content of calcium, magnesium and nitrogen per unit of fat free dry solid.

5. The pattern of change in body composition produced by potassium restriction is altered by administration of DCA. DCA causes a retention of sodium in the chloride space, thus greatly potentiating the alkalosis, but does not enhance potassium loss.

6. The extent of the potassium depletion could not be correlated with the gain in body sodium, with the changes in acid base balance or with the serum levels of potassium.

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THE DISTRIBUTION OF HG^{203}-LABELED MERCAPTOMERIN IN HUMAN TISSUES [1, 2]

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(Submitted for publication December 9, 1955; accepted March 15, 1956)

The availability of a radioactive isotope of mercury, Hg^{203}, and the recent improvements in instrumentation for radioactivity assay obviating loss of mercury through volatilization has made possible a further study of the behavior of an organic mercurial diuretic agent, mercaptomerin sodium (Thiomerin®). Previous studies in the rabbit (1) have shown that 87 to 96 per cent of the radioactivity administered as Hg^{203}-labeled mercaptomerin can be recovered in the urine, kidney, and liver. Analysis of the entire carcass, urine and feces of a rabbit 24 hours after injection of a tracer dose of Hg^{203} as labeled mercaptomerin showed that 97 per cent of the total administered radioactivity could be accounted for. Studies in the rabbit (1), rat and dog (2) have shown species differences in the renal excretion of mercury; residual radioactivity was concentrated primarily in the kidney. The concentration of mercury in the renal tissue varied considerably, even in normal healthy rabbits (1). This finding, together with the clinical observation that the diuretic response of human beings to the injection of a standard amount of a mercurial agent cannot be predicted, suggested that there might be considerable variation in the ability of diseased human kidneys to concentrate mercury administered in an organic form and that this variability may be one of the factors contributing to the erratic response to mercurial therapy.

The purpose of the present study was to determine the tissue distribution of Hg^{203} which had been administered parenterally as labeled mercaptomerin sodium,[4] and to compare its distribution in human tissues with that previously observed in rabbits.

Material. Hg^{203}-labeled mercaptomerin sodium was synthesized to contain, per milliliter, 40 mg. of mercury in 140 mg. of Thiomerin®, and 140 microcuries of radioactivity. The material was received in a frozen state and stored at − 20 C. until immediately prior to use.

Subjects. Patients who were terminally and critically ill and in whom, in the opinion of the house officers and attending staff, no contraindications existed for the injection of tagged mercaptomerin sodium, were given 1 or 2 ml. of the material intravenously or subcutaneously. The present report is based on the findings at autopsy in 9 cases. Pertinent clinical data have been summarized in Table I.

Methods. One to 2 gram samples of tissues obtained at autopsy were weighed in 1-dram glass vials, which were then stoppered tightly. These samples were assayed for radioactivity by gamma ray counting in the well of a Nuclear Model DS-3 scintillation counter which was attached to a Tracerlab Superscaler. The concentration of mercury per gram of wet weight of tissues was then calculated from the known specific activity of the tagged Thiomerin®.

RESULTS

Tissues were obtained from subjects who died between 1¼ and 576 hours (24 days) after the administration of tagged mercaptomerin (Table I). Selective concentration of Hg^{203} in the renal parenchyma had begun as early as 1¼ hours after subcutaneous administration of the tagged Thiomerin® (case 1). In this patient the concentration of mercury in one kidney was 7.55 micrograms per gram at a time when the serum concentration was 7.26 micrograms per milliliter. Ten hours after the intravenous administration of the labeled mercaptomerin (case 2), the renal concentration was three times that in the serum. Appreciable amounts of mercury were detected in the kidneys for as long as 144 and 576 hours after the administration of a single tagged dose.

[1] This study was supported in part by grants-in-aid from the American Heart Association and Wyeth Laboratories, and in part under a contract with the U. S. Atomic Energy Commission.

[2] Hg^{203} was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, on allocation from the U. S. Atomic Energy Commission.

[3] Established Investigator of the American Heart Association.

[4] Hg^{203}-labeled mercaptomerin sodium was synthesized by the Wyeth Institute for Medical Research.

TABLE I

Distribution of Hg^{203}-labeled mercaptomerin in human tissues

Case	Initials	Hg^{203}-Thiomerin® Dose (*mg. Hg*)	Route	Time to death (*hrs.*)	Primary disease	Renal lesion (Microscopic diagnosis)	Kidney weights (*gms.*) Right	Left
1*	LH	80	SC‡	1.25	Arteriosclerotic heart disease	Nephrosclerosis, arterial, moderate§	140	150
2†	CD	40	IV	10	Meningococcemia; meningococcic meningitis	Nephrosclerosis, arterial, mild§	130	190
3	NH	40	IV	24	Arteriosclerotic heart disease; anuria	Nephrosclerosis, advanced	90	85
4	EB	80	IV	36	Myocardial infarction, recent	Nephrosclerosis, arterial, mild§	165	150
5	DW	80	IV	36	Acute lymphatic leukemia	Left, pyelonephritis, chronic		60
						Right, compensatory hypertrophy	270	
6	JD	40	IV	48	Agranulocytosis; peritonitis, generalized	Normal	190	180
7	LS	80	SC	48	Carcinoma of pancreas	Pyelonephritis, chronic, mild	160	160
8	ER	80	IV	144	Rheumatic heart disease	Nephrosclerosis, arterial, minimal§	190	170
9	DL	40	SC	576	Cor pulmonale; duodenal ulcer with hemorrhage	Normal	145	160

* Case 1: Thyroid = 0.85 μg. Hg per gm.; bladder = 0.44 μg. per gm.
† Case 2: Bone = 0.74 μg. per gm.
‡ SC = subcutaneous injection; IV = intravenous injection.
§ Vascular changes considered by pathologist to be not unusual for age of patient and to not imply significantly altered renal function.

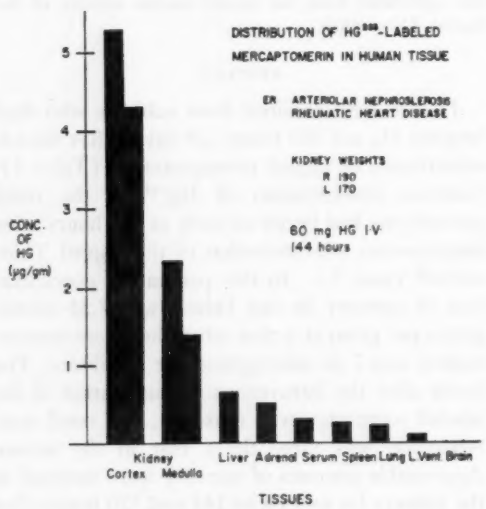

FIG. 1. CASE 8, TABLE I

Note the relative tissue distribution and the renal concentration of radioactivity. The cortex contained more radioactivity than the medulla.

In most instances, the concentration in the kidneys was approximately three times higher than that in the liver—the tissue with the next highest concentration. Tissue from cortex contained approximately twice as much mercury per gram as that from medulla (case 8, Figure 1). The concentration of mercury in brain and bone was negligible, and the values in the other tissues were intermediate between those in the kidney and those in brain and bone.

In general, the concentrations of mercury attained in the two kidneys were roughly equal; the one exception was in a patient (case 5, Figure 2) who at autopsy was found to have one pyelonephritic kidney weighing 60 gm. and one hypertrophic kidney weighing 270 gm. The concentration of mercury in the latter (26.16 micrograms per gram) was over twice that in the smaller organ (11.81 micrograms per gram). The highest renal concentration of mercury (69.86 micrograms per gram) was found in the subject (case 4) who

TABLE I—*Continued*

Concentrations of mercury (μg. Hg/gm. wet weight)									
Serum	Kidney	Adrenal	Skeletal muscle	Left ventricle	Lung	Spleen	Brain	Colon	Liver
7.26	6.33‖ 7.55	1.63	1.48	1.06	1.88	1.18	0.11	1.08	2.30
2.78	8.42 9.38			0.80	1.73	0.90	0.16		1.75
	1.92 1.24			0.80		0.98		1.23	2.18
	69.86				3.09				2.82
5.44	11.81	3.73	2.11	1.41	7.77	6.32			4.98
	26.16								
	6.82 5.10								
0.91	16.62				1.29	0.61		1.33	1.78
0.29	Cortex 5.31 4.30 Medulla 2.33 1.38	0.50		0.11	0.22	0.26	0		0.64
	9.03 6.45								0.90

‖ Values from both kidneys when available.

died of an acute myocardial infarction and in whom there was no history of previous renal disease and postmortem findings revealed only very mild arterial nephrosclerosis. The lowest renal concentrations (1.92 and 1.24 micrograms per gram) were found in a patient with far-advanced nephrosclerosis (case 3, Figure 3) who was anuric for the 24 hours preceding death. In this subject, 24 hours after injection of the Hg^{203}-labeled mercaptomerin, the mercury concentration in the kidneys was less than that in the liver.

COMMENT

Measurements of the total mercury content of the kidneys and other tissues at autopsy have been reported in previous studies on the renal toxicity of mercuric chloride (3) and organic mercurial diuretic agents (4, 5). The kinetics and localization of mercury administered as a single dose of diuretic can be traced by the use of the radioisotopic technique.

The results of the present radioisotopic study indicate that the renal concentration of mercury following the parenteral administration of one dose of tagged mercaptomerin varies widely in magnitude and duration in different subjects. The con-

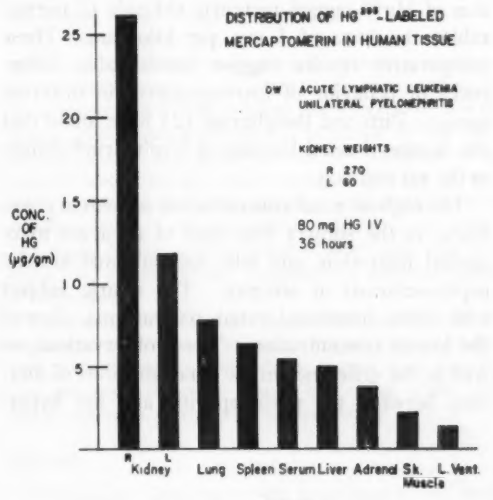

FIG. 2. CASE 5, TABLE I

The atrophic left kidney contained less radioactivity than the hypertrophied right kidney.

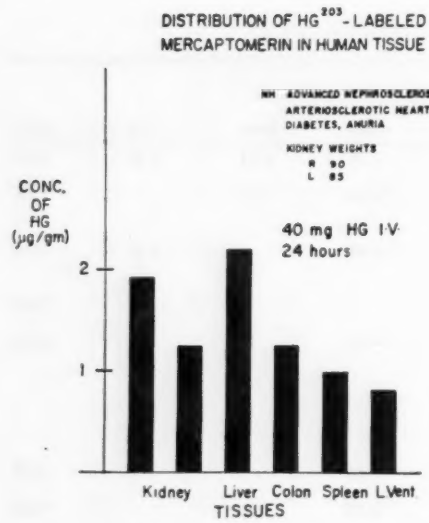

FIG. 3. CASE 3, TABLE I

Both kidneys were abnormally small and concentrated mercury least of the nine cases studied.

centrations of mercury in the various tissues within the first 48 hours after injection were as much as ten times higher than those observed previously in normal rabbits given an equivalent dose of labeled mercaptomerin (1), and approached the values reported by Adam (6) following the administration of Hg^{203}-tagged mercuric chloride to normal rabbits in doses of 1 mg. per kilogram. These comparative results suggest considerable differences of behavior of mercaptomerin in different species; Pitts and Borghgraef (2) have found that the degree of renal binding of Neohydrin® differs in the rat and dog.

The highest renal concentration of mercury was found in the subject who died of an acute myocardial infarction, and who had minimal arterial nephrosclerosis at autopsy. The anuric subject with little functional renal parenchyma showed the lowest concentration. These observations, as well as the difference in the concentrations of mercury between the pyelonephritic and the hypertrophied kidneys of patient 5, suggest that the ability of the organ to concentrate mercury depends at least partially upon the quantity and quality of the functional tissue. The higher concentration of mercury in the renal cortex, as compared with the medulla, is consonant with the view that the organic mercurial agents localize primarily in the distal or proximal tubules (7).

SUMMARY

Forty to 80 mg. of mercury as Hg^{203}-labeled mercaptomerin sodium was administered parenterally to 9 terminally ill patients, and tissue analyses were performed 1¼ to 576 hours later at autopsy. In all cases the kidneys were found to contain mercury. The highest concentration was 70 micrograms per gram of wet tissue; most of the values ranged between 1.2 and 9.4 micrograms per gram. The concentration appeared to be dependent upon the quantity and the quality of the renal parenchyma.

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TOTAL BODY WATER AND TOTAL EXCHANGEABLE SODIUM IN EDEMATOUS STATES DUE TO CARDIAC, RENAL OR HEPATIC DISEASE [1]

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(Submitted for publication August 25, 1955; accepted March 15, 1956)

The physiological defects of salt and water retention appear to be the same in diseases of various etiologies associated with edema. However, the relative amounts of sodium and water retained and their distribution may be different and related to the metabolic faults of the particular disease. Although the concentrations of electrolytes in extracellular fluid are similar in edematous states, the concentrations in other compartments may indicate differences related to the relative accumulation and distribution of sodium and water.

At present, methods are available to determine the concentrations of electrolytes in extracellular fluid. In intact man, concentrations of ions in other compartments of the body have to be indirectly determined. One such assay may be attempted by computing the body concentration of the electrolyte from the determinations of total exchangeable body content of the electrolyte and total body water. The value so derived does not have the physical chemical significance of concentration but may be indicative of changes occurring in the extracellular fluid as well as in other body compartments.

Employing this procedure, the present study attempts to differentiate various clinical conditions with edema on the basis of total exchangeable sodium and body water content. In particular, patients with congestive heart failure have been compared to patients with other diseases associated with edema. The data indicate that patients with congestive heart failure apparently have a high body concentration of sodium (mEq. of exchangeable sodium per liter of body water). This increase in the ratio of body sodium to body water persists in the majority of patients with heart failure after they have been rendered free of edema. A difference also exists between edematous patients with heart disease and edematous patients with hepatic or renal disease in body water and body water expressed as a per cent of body weight. The patients with edema due to heart disease have less body water and body water as the per cent of body weight than do edematous patients with liver or renal disease.

[1] Major support for this study came from a grant (H-1270) of the United States Public Health Service, National Heart Institute. Additional support was given by the Life Insurance Medical Research Fund, the Atomic Energy Commission and some of the work was done under the sponsorship of the Commission on Hemorrhagic Fever.

SUBJECTS AND METHODS

Subjects

The control group studied consisted of 27 patients without edema and with no evidence of renal, liver or heart disease. Among these subjects were 5 patients who had recent marked reduction in weight due to neoplastic disease. These 5 patients were intended to serve as a control group for heart disease patients who may have lost weight before or during the study.

The heart disease group consisted of 50 patients with edema and 33 patients who were previously edematous. Of the latter group of 33 subjects, 13 were studied in the edematous state and restudied when they were free of edema. They were rendered free of edema by bed rest, digitalis and/or mercurial diuretics and remained at a constant weight for at least 3 weeks. The other 20 patients in this non-edematous category were clinically free of edema for a period of several months to one year. The heart disease patients included individuals with hypertensive, arteriosclerotic, rheumatic or unknown types of heart disease.

The hepatic and renal groups studied consisted of 16 subjects with edema and/or ascites due to cirrhosis of the liver and 10 subjects with edema due to renal disease. The patients with renal disease had either acute glomerulo-nephritis or the nephrotic syndrome.

Methods

Body water. Body water was determined by the antipyrine dilution method (1). After drawing blood for a blank serum determination, 20 ml. of 5 per cent antipyrine were injected from a calibrated syringe. In the non-

TABLE I

Comparison of body water as determined by dilution of antipyrine and deuterium in edematous patients with heart disease

Patient	Antipyrine space (*Liters*)	Deuterium space (*Liters*)
1	45.3	47.2
2	49.8	51.0
3	64.0	62.8
4	51.5	50.0
5	33.5	35.9
6	47.2	48.2
7	38.2	40.8
8	50.6	50.6
9	41.0	41.8
Mean	46.8	47.6

edematous patients blood samples were drawn 5, 7 and 9 hours after the injection of antipyrine. In the patients with edema, specimens of blood were drawn 7, 9, 11, 13, and in some instances 18 and 24 hours after the injection of antipyrine. The concentrations of antipyrine were determined by accepted chemical procedures (2).

TABLE II

Concentration of antipyrine in plasma and extravascular fluids *

Fluid source	Number	Ratio of $\frac{\text{Fluid concentration}}{\text{Plasma concentration}} \times 100$ Mean	Range
Edema	16	98.4	83–111
Ascitic	5	99.3	94–103
Pleural	2	102.0	

* These values were determined on samples taken 10 to 24 hours after the injection of antipyrine.

The validity of the antipyrine method for the determination of body water in normals and patients with abnormal fluid accumulation due to liver disease has been established in other studies (1, 3). Since a reduced rate of blood flow may hamper equilibration in body fluids of any injected substance it was necessary to validate the

TABLE III

Exchangeable sodium and body water in patients without clinical evidence of heart, renal or hepatic disease—"controls"

Patient	Weight (*Kilos*)	Serum sodium (*mEq./L.*)	Antipyrine space (*Liters*)	Total exchangeable sodium (*mEq.*)	$\frac{\text{A.S.}}{\text{Wt.}}$* (% *Body wt.*)	$\frac{\text{TENa}}{\text{A.S.}}$* (*mEq./L.*)	$\frac{\text{TENa}}{\text{Wt.}}$* (*mEq./Kilo*)
1	68.2	139	35.2	2,840	51.6	80.8	41.7
2	60.9	144	31.1	2,600	51.1	83.6	42.7
3	54.1	134	35.0	2,550	64.6	73.0	47.1
4†	38.6	136	27.4	2,320	71.0	85.0	60.1
5	69.6	136	36.5	3,280	52.4	88.5	47.1
6	68.6	136	45.5	2,920	65.0	64.0	42.6
7	70.5	127	34.0	2,480	48.5	73.0	35.2
8	81.8	144	35.0	3,030	42.8	86.6	37.1
9†	55.0	135	32.6	3,050	41.5	93.0	55.5
10	80.9	148	40.5	3,240	50.0	80.0	40.1
11	98.1	132	41.1	3,630	41.9	88.5	37.0
12	74.6	138	40.7	3,310	54.6	81.4	44.4
13	71.9	137	38.6	3,060	53.6	79.2	42.6
14	79.1	142	48.6	3,470	61.5	71.4	43.9
15	37.0	140	25.3	2,130	68.4	84.0	57.6
16	57.3	139	26.3	2,100	45.9	80.0	36.7
17	101.5	141	48.0	3,950	45.5	78.0	38.9
18	71.5	130	33.4	2,700	46.8	80.8	37.8
19	71.5	135	35.5	2,760	49.6	77.8	38.6
20†	59.5	142	29.1	2,540	49.0	87.5	42.7
21†	52.4	145	28.8	2,590	55.0	90.0	49.5
22†	59.5	131	33.5	2,790	56.3	83.3	46.9
23	80.5	141	42.5	3,440	52.9	81.0	42.7
24	68.7	134	36.8	3,160	53.6	86.0	46.0
25	47.3	137	26.1	2,040	55.2	78.2	43.2
26	55.0	141	31.5	2,550	57.3	82.0	46.4
27	73.2	141	35.3	2,080	48.2	75.9	36.6
Mean	66.8	138	35.0	2,896	52.0	81.3	43.7
Standard deviation	15.3	4.9	7.0	479	6.3	6.2	5.9
Standard error	3.0	0.99	1.2	94	1.3	1.2	1.2

* In all tables: A.S.—Antipyrine Space, TENa—Total Exchangeable Sodium, TENa/AS—Exchangeable Sodium per liter of Antipyrine Space (Body Water), TENa/Kg.—Exchangeable Sodium per kg. Body Weight.

† Patients with recent loss of weight due to neoplastic disease.

TABLE IV

Exchangeable sodium and body water in patients with edema due to heart disease

Patient	Weight (*Kilos*)	Serum sodium (*mEq./L.*)	Antipyrine space (*Liters*)	Total exchangeable sodium (*mEq.*)	A.S./Wt. (% *Body wt.*)	TENa/A.S. (*mEq./L.*)	TENa/Wt. (*mEq./Kilo*)
1	60.6	133	33.8	3,900	55.8	115.5	64.4
2	91.0	138	43.9	4,450	48.3	101.0	49.0
3	94.1	135	47.3	5,000	55.6	105.5	53.2
4	70.3	140	43.9	4,300	62.5	98.0	61.2
5	75.4	133	49.6	4,800	65.8	97.4	63.7
6	60.6	144	33.5	3,270	55.3	97.5	55.7
7	66.4	137	30.5	2,910	45.9	95.5	43.7
8	66.8	140	41.7	4,020	62.4	96.0	60.2
9	70.3	140	37.3	4,735	55.9	102.0	67.4
10		140	33.2	3,450		104.0	
11	58.7	128	32.3	3,560	55.0	110.0	60.7
12	65.0	130	36.8	4,620	56.6	125.0	71.1
13	51.4	141	33.7	3,880	65.7	115.0	75.5
14	68.2	136	39.3	4,080	57.7	102.0	59.8
15	45.5	128	25.1	2,840	55.1	110.0	62.4
16	74.6	136	50.1	4,405	67.2	88.7	59.1
17	50.0	130	27.0	2,720	54.0	100.6	54.4
18	65.0	138	34.0	3,690	52.3	108.5	56.8
19	110.5	131	62.1	7,050	56.2	113.5	63.8
20	66.0	145	43.5	6,100	66.0	140.0	92.5
21	58.2	140	36.2	4,060	62.2	112.0	69.8
22	68.2	134	33.0	3,620	48.4	110.0	53.1
23	60.0	135	24.8	2,585	41.4	104.6	43.1
24	82.0	142	42.0	4,150	51.2	98.8	50.6
25	64.6	138	35.6	4,445	55.1	125.0	68.9
26	64.5	142	30.5	4,090	47.3	134.0	63.4
27	68.8	133	35.7	4,590	51.9	128.5	66.8
28	56.0	139	32.9	3,515	58.8	106.7	62.8
29	64.0	141	29.3	3,640	45.8	124.0	56.9
30	63.5	137	30.5	3,170	47.6	104.0	49.9
31	71.0	143	44.3	4,730	62.4	106.5	66.6
32		140	29.5	3,030		102.5	
33	55.0	141	31.6	4,000	57.4	115.6	72.8
34	77.0	144	37.9	3,790	49.2	100.0	49.2
35	58.5	135	33.1	3,500	56.5	105.6	59.9
36	72.6	140	25.5	2,580	33.4	101.0	35.5
37	85.0	132	47.6	4,761	56.0	100.0	56.0
38	71.0	127	50.6	5,100	71.3	105.0	71.9
39	59.2	140	25.4	2,800	43.0	110.0	47.4
40	69.5	139	44.8	5,220	50.0	116.5	75.1
41	86.9	138	49.8	5,080	57.4	102.0	58.4
42	68.7	133	44.0	4,350	64.1	99.0	63.3
43	75.0	136	32.3	2,690	43.1	83.3	35.9
44	62.3	136	34.8	4,170	55.8	119.5	66.9
45	65.9	135	38.0	4,650	57.6	124.0	70.6
46	64.6	127	34.1	3,390	52.8	99.5	52.5
47	104.0	128	52.0	5,510	50.0	106.0	53.0
48		141	27.5	3,850		140.0	
49	62.9	136	33.5	3,760	53.3	112.0	59.8
50		125	33.5	3,200		96.0	
Mean	68.2	136	37.9	4,036	55.5	108.2	59.9
Standard deviation	12.9	5.0	8.26	933	7.5	12.0	11.0
Standard error	1.93	0.7	1.18	133.4	1.41	1.7	1.6

antipyrine dilution method as a measure of body water in patients with edema due to congestive heart failure. Accordingly, simultaneous determinations of total body water by deuterium and antipyrine dilution were performed in nine markedly edematous patients with congestive heart failure. Deuterium was administered intravenously by a pump with a burette as a reservoir. Serum concentration was determined by the mass spectrometer.[2]

As a further aid in establishing the antipyrine method as a measure of body water in patients with abnormal fluid accumulations, equilibration of antipyrine between

[2] We are indebted to Mr. Irving Sucher of the Department of Chemistry, College of Physicians and Surgeons, for these determinations.

blood and large extravascular collections of fluid was determined. These fluids consisted of 23 samples of edema, ascitic and pleural fluid studied at various intervals after the injection of antipyrine.

The sources of the extravascular fluids studied were as follows: 16 edema fluids: 14 from patients with heart disease, one from a patient with cirrhosis of the liver, and one from a patient with edema of unknown cause; 5 ascitic fluids: one specimen from a patient with heart disease and four from patients with cirrhosis of the liver; 2 pleural fluids: a specimen from a patient with heart disease and one from a patient with renal disease.

Total exchangeable sodium. Total exchangeable sodium was determined by the dilution of radioisotopic sodium (4–6). One ml. of Na^{24} containing 100 microcuries was injected from a tuberculin syringe. Twenty-four hours were allowed for mixing and equilibration of the injected radioactive element with the exchangeable body sodium pool. Twenty-four and twenty-six hours after the injection samples of blood were drawn and serum analyzed. If these determinations did not agree within 5 per cent, a third and if necessary a fourth sample was drawn at hourly intervals until the concentration remained constant. Specimens of edema, ascitic and pleural fluid drawn twenty-four hours after injection of Na^{24} were compared for radioactivity with serum drawn at the same time.

In most instances the loss of radiosodium in the urine was not over one per cent of the administered sodium. When this value was above one per cent, it was subtracted from the injected dose.

Serum sodium was determined by the use of an internal standard flame photometer.

RESULTS

Comparison of body water by antipyrine and deuterium dilution methods in patients with congestive heart failure

The spaces of distribution of antipyrine and deuterium were very similar, antipyrine 46.8 liters,

TABLE V

Exchangeable sodium and body water in patients with heart disease who were previously edematous

Patient	Weight (*Kilos*)	Serum sodium (*mEq./L.*)	Antipyrine space (*Liters*)	Total exchangeable sodium (*mEq.*)	A.S./Wt. (*% Body wt.*)	TENa/A.S. (*mEq./L.*)	TENa/Wt. (*mEq./Kilo*)
1	36.4	138	27.4	2,260	75.3	82.5	62.1
2	65.0	132	42.1	3,370	64.8	80.0	51.9
3	66.8	140	37.3	3,050	55.0	82.0	45.7
4	76.4	140	36.2	3,920	47.4	108.0	51.3
5	61.6	139	37.8	3,060	61.5	81.0	49.7
6	77.3	140	37.2	4,660	48.1	125.0	60.3
7	53.6	136	27.0	2,650	50.2	97.5	49.5
8	78.7	139	43.5	4,395	55.1	101.0	55.9
9	66.9	140	29.0	3,472	43.4	120.5	51.9
10	49.1	134	24.1	2,420	49.1	100.5	49.4
11	61.8	140	28.2	2,800	45.7	99.6	45.3
12	66.4	141	30.2	3,370	45.5	112.0	50.7
13	63.7	135	31.8	2,620	49.8	86.4	41.1
14	70.0	137	41.1	3,540	58.7	86.4	50.6
15	61.3	137	43.0	3,700	70.2	86.0	60.4
16	77.8	134	41.3	3,850	53.1	92.2	49.5
17	47.7	141	29.2	2,670	61.3	91.5	55.9
18	56.4	141	30.9	3,170	54.9	103.0	56.3
19	77.3	138	38.5	3,630	49.8	94.4	46.9
20	58.2	138	36.1	2,940	62.0	98.1	50.6
21	63.8	140	32.0	3,680	51.3	115.0	57.7
22		147	22.6	2,295		101.5	
23	54.5	138	25.0	2,630	45.8	105.0	48.3
24	61.3	141	30.6	3,350	49.8	109.5	54.6
25	74.1	140	37.2	3,880	50.2	104.0	52.4
26	52.3	140	32.0	3,360	61.2	105.0	64.2
27	51.4	135	32.4	2,760	62.9	85.5	53.7
28	69.0	140	25.0	2,730	36.2	109.0	39.6
29	57.7	135	31.8	3,595	55.1	114.0	62.3
30	52.0	139	27.0	3,060	51.9	113.4	58.8
31	109.5	140	56.2	5,760	51.3	102.5	52.5
32	60.0	142	35.8	3,950	59.5	110.0	65.7
33	61.5	138	38.2	4,360	62.2	114.0	70.9
Mean	63.7	139	34.2	3,362	54.2	100.4	53.5
Standard deviation	12.8	2.9	7.14	722	7.8	12.1	6.7
Standard error	2.3	0.5	1.3	128	1.4	2.1	1.2

TABLE VI

Exchangeable sodium and body water in patients with hepatic disease with edema and/or ascites

Patient	Weight (*Kilos*)	Serum sodium (*mEq./L.*)	Antipyrine space (*Liters*)	Total exchangeable sodium (*mEq.*)	A.S./Wt. (*% Body wt.*)	TENa/A.S. (*mEq./L.*)	TENa/Wt. (*mEq./Kilo*)
1	54.6	128	36.5	2,830	65.9	78.0	51.8
2		130	33.4	2,710		81.4	
3		126	37.8	3,400		90.0	
4	85.5	130	51.4	4,770	60.1	96.0	55.8
5	86.2	140	53.0	6,120	61.5	115.0	71.0
6	53.2	128	36.5	3,000	68.6	82.5	56.4
7	55.0	136	37.2	3,130	67.7	84.0	57.0
8	74.2	131	50.4	3,820	67.9	76.0	51.5
9	68.2	130	43.4	3,620	63.6	83.5	53.0
10	88.0	128	59.7	5,940	67.8	98.0	67.5
11	111.0	134	46.0	4,320	41.4	94.0	39.3
12	78.5	124	45.5	4,080	58.0	89.0	51.9
13	48.0	125	27.0	2,070	56.2	78.0	43.2
14	72.0	133	46.5	4,210	64.6	90.0	58.5
15	56.0	130	37.0	3,380	66.0	91.0	60.4
16	50.5	122	32.2	3,600	63.8	117.0	71.4
Mean	70.0	130	43.0	3,810	61.0	90.3	56.2
Standard deviation	14.5	4.6	8.82	1,097	7.15	11.6	9.3
Standard error	4.0	1.18	2.3	284	1.98	3.0	2.6

deuterium 47.6 liters, in the same edematous patients with heart disease (Table I). Hence, in patients with congestive heart failure and varying amounts of edema, antipyrine would appear to be a valid measure of body water.

Degree of equilibration of antipyrine between plasma and extravascular fluid

In most instances the concentration of antipyrine in plasma and in samples of large accumulations of extravascular fluid collected 10 to 24 hours after the injection of antipyrine was in good agreement (Table II).

Exchangeable sodium in extravascular fluids

On examining 13 of the sera and extravascular fluids for radioactivity, it was found that, at the end of 24 hours, each of the fluids had the same concentration of radioactivity as the simultaneously drawn serum.

TABLE VII

Exchangeable sodium and body water in patients with renal disease with edema

Patient	Weight (*Kilos*)	Serum sodium (*mEq./L.*)	Antipyrine space (*Liters*)	Total exchangeable sodium (*mEq.*)	A.S./Wt. (*% Body wt.*)	TENa/A.S. (*mEq./L.*)	TENa/Wt. (*mEq./Kilo*)
1	65.6	142	51.4	4,760	78.3	92.6	72.6
2	82.3	139	62.2	5,660	75.5	91.0	68.7
3	58.2	136	28.7	2,585	49.2	89.9	44.4
4	73.7	137	49.0	4,390	66.5	89.6	59.5
5	52.7	136	32.3	2,870	61.3	91.0	54.5
6	82.4	137	52.8	3,960	64.1	75.0	48.1
7	56.9	138	28.6	2,590	50.2	90.8	45.5
8	60.4	134	44.2	3,860	73.2	87.5	64.0
9	60.9	136	42.4	4,020	69.6	94.8	66.1
10	65.0	132	40.0	3,590	61.4	90.0	55.2
Mean	65.8	137	43.2	3,828	65.0	89.2	57.9
Standard deviation	10.7	2.72	11.1	975	9.85	5.1	9.9
Standard error	3.46	0.91	3.7	325	3.28	1.7	3.3

TABLE VIII

Summary of mean values

	Controls	Edematous cardiacs	Non-Edematous cardiacs	Hepatic disease	Renal disease
Weight (*Kilos*)	66.8	68.2	63.7	70.0	65.8
Antipyrine space (*Liters*)	35.0	37.9	34.2	43.0	43.2
A.S./Kilo (*% Body wt.*)	52.0	55.5	54.2	61.0	65.0
Total exchangeable sodium (*mEq.*)	2896	4036	3362	3810	3828
TENa/A.S. (*mEq./Liter*)	81.3	108.2	100.4	90.3	89.2
TENa/Kg. (*mEq./kilo*)	43.7	59.9	53.5	56.2	57.9
Number of subjects	27	50*	33*	16*	10

* In the edematous cardiac, non-edematous cardiac, and hepatic groups there were 4, 1, and 2 patients, respectively, whose weights were not obtainable.

Antipyrine space and antipyrine space as per cent of body weight

The edematous patients with hepatic or renal disease, had a significantly larger mean value for body water and body water expressed as per cent of body weight than any of the other groups (Tables VIII, XI C). The patients with edema due to heart disease had a small increase above normal that was not statistically different from the control. The group of patients with heart disease who were previously edematous, had a value closer to normal.

Total exchangeable sodium antipyrine space (Tables III to VIII)

In an effort to determine body sodium concentration, total exchangeable sodium and antipyrine space were related to each other. In the controls the resultant ratio expressed as milliequivalents of sodium per liter of body water was 81.3 with a standard deviation of 6.2 and a standard error of 1.2 (Table III). In each group of edematous patients with edema due to heart, renal or liver disease and in patients with heart disease who were previously edematous, the ratios of exchangeable sodium to body water were significantly higher than the controls (Table XI A).

The following mean values for the ratios were obtained: in patients with edema due to heart disease 108.2 (Table IV), hepatic disease 90.3 (Table VI), renal disease 89.2 (Table VII), and in treated heart disease patients who had lost their edema 100.4 (Table V, Figures 1, 2, and 3). The values for each of the four abnormal groups were significantly different from each other except that the value obtained in patients with edema due to renal disease was not significantly different

TABLE IX

Comparison of exchangeable sodium and body water in patients with heart disease when edematous and later when non-edematous *

Edematous patient	Weight (*Kilos*)	Antipyrine space (*Liters*)	A.S./Wt. (*% Body wt.*)	TENa/Kg. (*mEq./Kg.*)	TENa/A.S. (*mEq./L.*)	Patient	Non-Edematous Weight (*Kilos*)	Non-Edematous Antipyrine space (*Liters*)	Non-Edematous A.S./Wt. (*% Body wt.*)	Non-Edematous TENa/Kg. (*mEq./Kg.*)	Non-Edematous TENa/A.S. (*mEq./L.*)
2	91.0	43.9	48.3	49.0	101.0	14	70.0	41.1	58.7	50.6	86.4
5	75.4	49.6	65.8	63.7	97.4	15	61.3	43.0	70.2	60.4	86.0
8	66.8	41.7	62.4	60.2	96.0	3	66.8	37.3	55.9	45.7	82.0
19	110.5	62.1	56.2	63.8	113.5	16	77.8	41.3	53.1	49.5	92.2
20	66.0	43.5	66.0	62.5	140.0	17	41.7	29.2	61.3	55.9	91.5
26	64.5	30.5	47.3	63.4	134.0	23	54.5	25.0	45.8	48.3	105.0
27	68.8	35.7	51.9	66.8	128.5	24	61.3	30.6	49.8	54.6	109.5
33	55.0	31.6	57.4	72.8	115.6	26	52.3	32.0	61.2	64.2	105.0
37	85.0	47.6	56.0	56.0	100.0	25	74.1	37.2	50.2	52.4	104.0
38	71.0	50.6	71.3	71.9	105.0	32	60.0	35.8	52.7	65.8	110.0
40	69.5	44.8	50.0	75.1	116.5	29	57.7	31.8	55.1	62.3	114.0
44	62.3	34.8	55.8	66.9	119.5	30	52.0	27.0	51.9	58.8	113.0
49	62.9	33.5	53.3	59.8	112.0	11	61.8	28.2	45.7	45.3	99.6
Mean	72.2	42.3	57.0	64.0	113.7		61.2	33.9	54.6	54.6	99.9

* These are the same patients. The patient number corresponds to the numbers in Tables IV and V.

TABLE X

Exchangeable sodium and body water in a patient with heart disease followed for five months

Date	Edema	Wt. (*Kg.*)	Antipyrine space (*Liters*)	TENa (*mEq.*)	TENa/A.S. (*mEq./Liter*)
11/18	++	68.6	35.3	4,590	129
12/11	0	61.3	32.3	4,220	130
1/5	0	61.4	30.6	3,350	109
3/16	0	61.4	32.2	2,880	89
4/28	0	65.0	32.9	2,600	79

than the one obtained in patients due to hepatic disease (Table XI A).

Of 33 non-edematous patients with heart disease, eight had ratios of total exchangeable sodium to antipyrine space which were in the normal range (Table V). The average of the ratios for the entire group was high, 100.4 and significantly different from that of the other groups of patients and the control group (Table XI A). There was no relationship between the value for the TENa/

TABLE XI

Statistical analyses

	Control	Edema due to heart disease	Previously edematous with heart disease	Edema due to: Hepatic disease	Edema due to: Renal disease
A) TENa/A.S. p (Probability)					
Control (N = 27)		>0.01	>0.01	>0.01	>0.01
Patients with edema due to:					
Heart disease (N = 50)	>0.01		>0.01	>0.01	>0.01
Hepatic disease (N = 16)	>0.01	>0.01	>0.01		0.50
Renal disease (N = 10)	>0.01	>0.01	>0.01	0.50	
Heart disease previously edematous (N = 33)	>0.01	>0.01		>0.01	>0.01
B) TENa/Kg. p (Probability)					
Control		>0.01	>0.01	>0.01	>0.01
Patients with edema due to:					
Heart disease	>0.01		>0.01	0.50	0.50
Hepatic disease	>0.01	0.50	0.50		0.50
Renal disease	>0.01	0.50	0.50	0.50	
Heart disease previously edematous	>0.01	>0.01		0.50	0.50
C) A.S. & A.S./Kg. p (Probability)					
Control		0.50	0.50	>0.01	>0.01
Patients with edema due to:					
Heart disease	0.50		0.50	>0.01	>0.01
Hepatic disease	>0.01	>0.01	>0.01		0.50
Renal disease	>0.01	>0.01	>0.01	0.50	
Heart disease previously edematous	0.50	0.50		>0.01	>0.01

D) Comparison of Heart Disease, Previously Edematous to Control

	Previously edematous with heart disease (N = 33)	Control (N = 27)	p
Weight	63.7	66.8	0.50
Antipyrine space	34.2	35.0	0.50
Total exchangeable Na	3362	2896	>0.01
TENa/A.S.	100	81	>0.01
TENa/Kg.	53	44	>0.01

E) Patients with Edema due to Heart Disease Compared to Patients with Cirrhosis with Similar Amounts of Body Water as Per Cent of Body Weight

	Number	A.S. as % of body weight	TENa/Kg.	p
Patients with edema due to:				
Heart disease	14	61.7	65.2	0.02
Hepatic disease	14	62.4	56.2	

TABLE XII

Theoretical calculations

	Weight (*Kg.*)	Body water (*Liters*)	% Body weight	TENa
Control: Serum Na = 140 mEq./L. (Assumed for all calculations)	67.0	35.0 TENa/A.S. = 82.5 TENa/Kg. = 43.7	53.0	2,896
I. Loss of 10 Kg. of weight in form of protein or 7.5 liters of intracellular water loss	57.0	27.5 TENa/A.S. = 105.0 TENa/Kg. = 50.9	48.2	2,896
II. A) Gain of 10 liters of extracellular water	77.0	45.0 TENa/A.S. = 95.2 TENa/Kg. = 55.8	58.5	4,296
B) Gain of 15 liters of extracellular water	82.0	50.0 TENa/A.S. = 100.0 TENa/Kg. = 61.0	61.0	4,996
III. Loss of 10 Kg. of weight in form of fat	57.0	33.0 TENa/A.S. = 88.0 TENa/Kg. = 51.0	58.0	2,896
IV. Loss of 10 Kg. of weight in form of protein or 7.5 liters of intracellular water plus gain of 10 liters of extracellular fluid	67.0	37.5 TENa/A.S. = 114.0 TENa/Kg. = 64.0	56.0	4,296

A.S. ratio obtained and the length of time a particular patient was edema free.

Body water measurements in this group of non-edematous patients with heart disease were not significantly different from the normal values. Exchangeable sodium proved to be significantly greater (Table XI D).

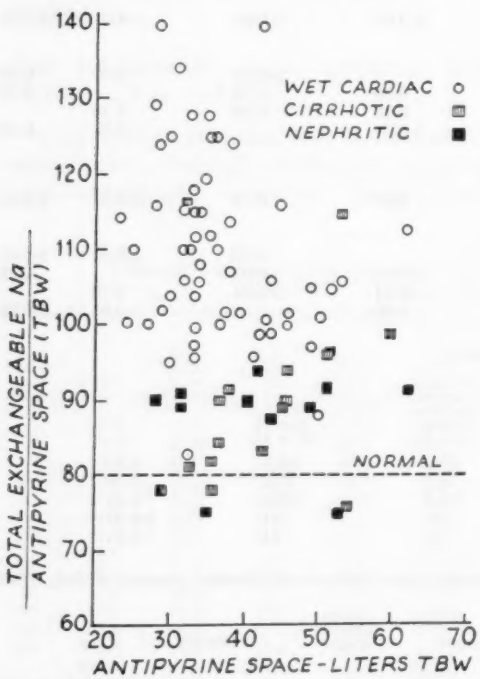

FIG. 1. TENa/A.S. IN EDEMATOUS PATIENTS

TBW = Total Body Water.

Two patients with renal disease had a ratio of body sodium to body water that returned to the normal range after a diuresis and when they were free of edema.

These data, therefore, indicate that patients with edema due to heart disease had a higher mean ratio of sodium to water in their bodies than did patients with excess of fluid accumulation due to renal or liver disease. This high ratio of sodium to water persisted in the bodies of the majority of patients with heart disease after their edema was gone.

Exchangeable sodium and body water in patients with heart disease before and after diuresis (Table IX)

Total exchangeable sodium and water were studied in 13 patients with edema due to heart disease and again when there was no clinical edema and their weights were constant for at least three weeks. The average weight decreased 9.0 kg., body water 8.4 liters, TENa per liter of body water 13.8 mEq., and TENa per kilogram body weight 9.4 mEq. The final values for TENa per liter body water and TENa per kg. body weight were

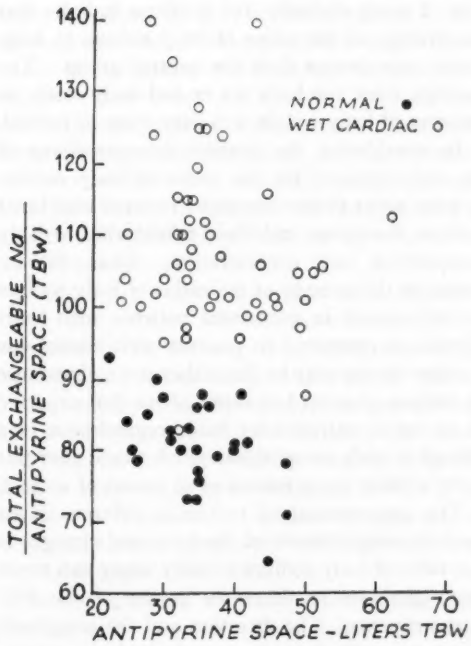

FIG. 2. TENa/A.S. IN PATIENTS WITH EDEMA DUE TO HEART DISEASE AND IN NORMALS

TBW = Total Body Water.

still significantly elevated as compared with the control. TENa per liter, 99.9 with control 81.3, TENa per kg. 54.6 with control 43.7. The mean figures for weight loss and water loss were in good agreement. However, the individual data showed a good deal of variation and no consistent cause could be established to explain the lack of correlation of the weight and water data in individual patients.

Serial determinations of body water and total exchangeable sodium

In one edematous patient with heart disease serial determinations were made over a 5-month period (Table X). He lost his edema during the first three weeks of hospitalization. As was characteristic of other patients of this type, his TENa per liter of body water remained elevated, 130 mEq. per liter. During the subsequent five months he did not lose weight or body water but his exchangeable sodium steadily decreased (1620 mEq.) until the value reached the normal range. Since his body water did not increase, the gain in weight during the last month of observation was considered to be due to a probable gain in adipose tissue. Thus, the patient continued to lose sodium after he became edema free. The clinical evaluation of his edema free state was substantiated by the observations of constant figures for body water during the four months of study.

Body weight and total exchangeable sodium. Total exchangeable sodium/body weight (kilos) (Table III to VIII).

To substantiate the finding of the possible abundance of sodium in patient with heart disease an analysis was made of total exchangeable sodium per kilogram body weight.

An analysis of exchangeable sodium expressed in mEq. per kg. body weight gave the following results (Tables III to VII): control 43.7; edematous patients with heart disease 59.9; with hepatic disease 56.2; with renal disease 57.9; non-edematous patients with heart disease 53.5 mEq. per kg. The values for each group of the edematous sub-

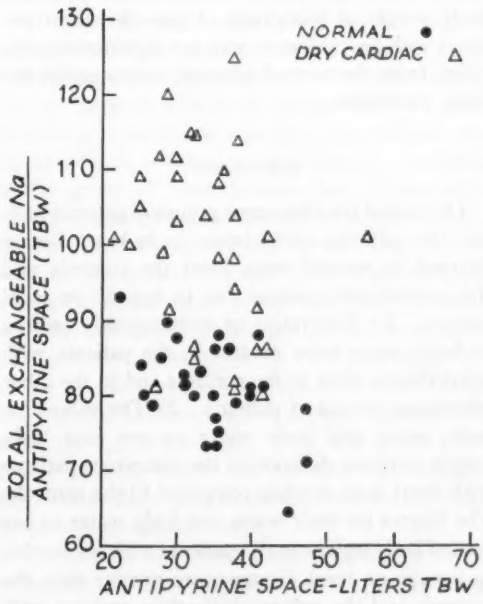

FIG. 3. TENa/A.S. IN PATIENTS WITH HEART DISEASE WHO WERE PREVIOUSLY EDEMATOUS AND IN NORMALS

TBW = Total Body Water.

jects differed significantly from the normal of 43.7 mEq. per kilo but not from each other (Table XI B).

Since body weight is the sum of various components which are variable from individual to individual, an attempt was made to compare individuals in the edematous group in whom body water composition, as per cent of body weight, was similar. For this purpose patients from the edematous group with heart disease were compared to patients with hepatic disease who had similar body water composition. When the values for sodium per kilo body weight of these chosen groups were then statistically analyzed, these groups were probably significantly different from each other. The p value of this analysis was 0.02 (Table XI E).

The patients with heart disease who were previously edematous and at the time of the study were edema free had a high average value for sodium per kilo body weight. When compared to the controls, the value for sodium per kilo body weight obtained was significantly greater. When compared to the values for the edematous patients with heart disease the ratio was lower (Table XI B). Body weight of this group of non-edematous patients with heart disease was not significantly different from the normal whereas exchangeable sodium was higher.

DISCUSSION

The data of the edematous groups of patients indicate that patients with edema due to heart disease differed in several ways from the controls and the patients with edema due to hepatic or renal disease. 1) The ratios of exchangeable sodium to body water were greater in the patients with heart disease than in the controls and in the other edematous groups of patients. 2) The values for body water and body water as per cent body weight were not different in the edematous patients with heart disease when compared to the controls. The figures for body water and body water as per cent of body weight in the patients with edema due to hepatic or renal disease were greater than the controls and the values obtained in patients with edema due to heart disease.

The data of the group of patients with heart disease who were previously edematous and at the time of study clinically free of edema indicate that the average of the ratios of body sodium to body water was greater than the control group. The average value for body water and body water as per cent of body weight was very close to normal.

In considering the possible interpretations of the data obtained for the ratios of body sodium to body water in the edematous patients with heart disease, the ratios and their relationship to body composition bear consideration. Thus, the increase in the average of the ratios of body sodium to body water in edematous patients with heart disease as compared to patients with edema due to other causes may be due either to an abundance of sodium as related to water above that expected to be due to extracellular fluid accumulation, or a change in body composition which would alter this ratio without the existence of an excess of sodium.

The concentration of sodium is different in the various compartments of the body and changes in the ratio of body sodium to body water can result from increases or decreases in any of the fluid compartments. The direction and the magnitude of change will depend upon the sodium concentration of the fluid gained or lost. These theoretical results are calculated in the accompanying list of Theoretical Calculations I–IV, Table XII. The calculations involving loss of body protein or fat assume values for water of these components derived by other investigators (7, 8).

Clinical edema is the result of the accumulation of an abundance of extracellular fluid. Since sodium exists in a concentration of approximately 140 mEq. per liter in extracellular fluid and in a lower concentration in cellular fluids, the accumulation of edema leads to a proportionately greater increase in total exchangeable sodium than in total body water. Theoretical Calculation II A shows this for a gain, as edema fluid, of 10 liters of extracellular water. An increase will result in the value for sodium per liter of body water. The estimated accumulation of edema in most of the patients with heart disease in this study was 10 to 12 liters. However, the individual values and the average of the ratios obtained in edematous patients with heart disease were much higher than the expected increases (averages: theoretical 95 mEq. per liter, 55.8 mEq. per kg.; observed 108 mEq. per liter, 60 mEq. per kg.). The patients with massive edema due to cardiac disease had

ratios far above 108. The high average of the ratios in the heart disease group could, therefore, be due to an increase of sodium above that anticipated as extracellular fluid edema. The renal and hepatic disease groups, exhibited the theoretical change expected in individuals with straightforward extracellular fluid accumulation of moderate severity. Values of this order of magnitude for patients with cirrhosis of the liver have been reported by others (9).

Loss of body weight may also affect sodium and water relationships. This would not occur if the loss were in the form of fat (Calculation III). However, if the loss was in the form of cellular protein and, assuming this to be 75 per cent sodium free water (8), this loss of intracellular water would produce a theoretical increase in body sodium to body water ratio (Calculation I). This value would increase still further if extracellular edema fluid were added to the individual with loss of cellular mass (Calculation IV).

The data obtained for body water indicate that patients with congestive heart failure probably lose intracellular water and gain extracellular water during the course of their illness. Individuals who just accumulate edema fluid should have an increase in body water but body water (expressed either as liters or as per cent body weight) did not increase in the edematous patients with heart disease. These findings support the probability that some loss of intracellular or other fluid almost balanced the simultaneous gain of extracellular water. Therefore, the observed high ratio of body sodium to water may be due to loss of cellular water and edema fluid accumulation, or to a combination of loss of cellular water, extracellular fluid accumulation and an additional excess of sodium.

In substantiating the occurrence of the combination of events in the patients with congestive heart failure, the data of the non-edematous group of patients with heart disease, indicating that they had an absolute abundance of sodium, are of considerable value. Several observations substantiate the conclusion concerning the existence of an excess sodium in these non-edematous patients.

1) The statistical analysis of the data of non-edematous heart disease group, as compared to the controls, showed that they differed only in that total exchangeable sodium was significantly greater (Table XI C). Body weight and body water were in the normal range. In the group of thirteen patients followed from the edematous to the non-edematous state a loss of water and sodium occurred, but an abnormally high ratio of body sodium to water persisted as a consequence of high total exchangeable sodium (Table IV). The increment in the average total exchangeable sodium in the whole group of non-edematous heart disease patients can be accounted for by a theoretical increase over the normal of four liters of extracellular fluid supplying an abundance of 560 mEq. of sodium. The presence of this amount of edema fluid could escape clinical detection. It may, therefore, be proposed that the high exchangeable sodium may be due to an increase of extracellular fluid that could not be observed on clinical examination. However, the value of 4 liters or 560 mEq. of sodium is the mean for the group and includes data of 8 subjects who fall in the normal range and the data of 12 subjects whose fluid accumulation on the basis of the same type of calculation would be between 5 and 10.4 liters. The latter orders of magnitude of extracellular fluid accumulation would have been evident as edema.

2) Since the body water data imply a loss of cellular water in patients with congestive heart failure, it would be anticipated that after edema is gone, patients with heart disease would be comparable to individuals who have lost cellular water as a result of other types of disease. Included in the group of controls were five patients with advanced malignancies. If loss of weight in neoplastic diseases is a result of cellular loss, the anticipated theoretical increase in the ratio of body sodium to water would be the result of a decrease in body water with no change in total exchangeable sodium (Calculation I). Only a small increase in the average ratio was observed (Table III) in the patients with malignant disease (87 mEq. per L., body water). This was due to a decrease in body water and exchangeable sodium. Apparently these individuals lost extracellular water as well as cellular water.

The data of the heart disease patients with no edema differ from the data obtained in the patients with malignant diseases. The non-edematous heart disease patients considered as a group as well as the thirteen patients followed from the edematous to the non-edematous phase of their

disease had no decrease in body water when compared to the controls but exchangeable sodium was increased. These observations indicate that intracellular water that was lost in the congestive phase of heart failure was replaced, and some retention of non-extracellular fluid sodium was still present.

3) The study on patient McL. (Table X) indicated that this patient with heart disease had a continued decrease in exchangeable sodium from a high to a normal level over a period of five months after edema had clinically disappeared and there was objectively no change in body weight and body water. This patient, therefore, had an excess of sodium and not of water when he reached the edema free status. Subsequently he lost his excess of sodium without changing his body water.

These data, therefore, suggest that the non-edematous patient with heart disease has an abundance of sodium and it would be reasonable to conclude that the high ratio of body sodium to water in the edematous state due to heart disease is not entirely the result of a loss of cellular water and simultaneous accumulation of extracellular fluid but is in part due to the existence of an absolute abundance of sodium over water.

The data obtained in the patients with hepatic or renal disease may be due to simple extracellular fluid accumulation or to a combination of extracellular and intracellular fluid accumulation and an excess of sodium. An increase in intracellular sodium poor fluid could decrease an otherwise high ratio of body sodium to water. This possibility cannot be ruled out with the present data. The observations that two patients with renal disease had normal ratios when they recovered would suggest that extracellular fluid accumulation alone was present in their edematous state.

The conclusion concerning an excess of sodium in patients with heart disease would receive further support if the total sodium to body weight ratio in these individuals was as significantly elevated as the total sodium to body water ratio. The ratio of body sodium to body weight of the non-edematous patients with heart disease proved to be statistically different from the other groups of patients.

In the edematous patients with heart disease the total sodium to body weight ratio was not statistically different from the ratios of the other edematous groups. The use of body weight as a standard of reference in this type of analysis is not ideal. Body weight is the sum of weights of different tissues and fluids of the body and variations in these components (*e.g.*, fat) may occur which are not related to water or sodium. The edematous groups of patients in this study were heterogeneous in their body weights and not enough patients with hepatic or renal disease were studied to make this heterogeneity comparable in all the three edematous states. For this reason, patients from hepatic and heart disease groups who had similar amounts of body water as a per cent of body weight were separately compared to each other in regard to their sodium to body weight ratio. The results of this comparison (Table XI D) indicated that the difference between these two groups of edematous patients was probably significant.

Some questions may be raised concerning the methods used in investigating these groups of patients. Validation of the antipyrine space as a measure of body water in patients with edema due to heart disease, is provided by the deuterium dilution data and extravascular fluid equilibrium data. It would be expected that the accumulation of edema would lead to an increase in water as a per cent of body weight. This was true of the edematous patients with liver or renal disease. The edematous patients with heart disease, however, had only a slight increase. The latter observation has been made by others using tritium as a measure of body water (3).

Twenty-four-hour exchangeable sodium is probably a measure of most of the sodium in the body. All the sodium in bone does not equilibrate in twenty-four hours with radiosodium (10, 11). However, this unequilibrated portion of sodium may not be important in the physiological sodium pool of the body. To explain the data presented on the basis of some artefact in the measurement of exchangeable sodium, it would have to be postulated that in the patient with heart disease there is a pool of sodium which becomes exchangeable, and is not exchangeable in the normal or the patient with renal or hepatic disease. This possibility seems remote but cannot be ruled out.

The foregoing data and considerations indicate that patients with heart disease who have edema

retain more sodium than water in their bodies as compared to patients with edema due to liver or renal disease. Patients with heart disease in contrast to patients with edema due to hepatic or renal disease probably lose intracellular water as they gain extracellular water. Individuals with heart disease store excess sodium for a variable period of time after they are edema free. An explanation for the defect concerning sodium distribution in patients with heart disease may be sought amongst the following: 1) These individuals have exchanged extracellular sodium for some other intracellular ion and have thereby retained sodium in excess of their total body water. 2) Excess sodium has been stored in some depot such as bone, cartilage or connective tissue. 3) Sodium may be bound to an extracellular (12) or intracellular tissue constituent, a situation in which it would be exchangeable but would not exert the anticipated osmotic effect for sodium in these biological fluids.

SUMMARY

1. Body water and total exchangeable sodium have been determined in normals, patients with edema due to heart, renal or hepatic disease and in patients with heart disease who were previously edematous.

2. Body water was determined with antipyrine. The results obtained with antipyrine were compared to body water determined with deuterium in nine edematous patients with congestive heart failure and were found to be in very close agreement.

3. Edematous patients with heart disease have less body water and body water as a per cent of body weight than do patients with edema due to renal or hepatic disease indicating a probable loss of intracellular along with a simultaneous gain of extracellular water.

4. Edematous patients with heart disease have a higher ratio of body sodium to body water in their bodies than do edematous patients with hepatic or renal disease which is considered to result from a loss of intracellular water, gain of extracellular water, and an absolute increase in body sodium. This conclusion is supported by the observation that an excess of body sodium over body water persists in patients with heart disease who have been rendered edema free.

5. The data imply the existence of different metabolic defects concerning sodium and water distribution in these clinical conditions associated with edema formation.

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THYROXINE METABOLISM IN THE NEPHROTIC SYNDROME[1]

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(Submitted for publication October 31, 1955; accepted March 23, 1956)

The presence of hypometabolism and hypercholesterolemia in nephrosis suggests decreased thyroid function. Because of these abnormalities Epstein (1) treated a number of nephrotic patients with large doses of thyroid, and found that hypermetabolism could not be produced with daily doses of 4 to 5 grams of desiccated thyroid.[3] Leiter, therefore, suggested (2) that the low basal metabolic rates and high concentrations of serum cholesterol in this disease were due to some cause other than hypothyroidism. The demonstration of low concentrations of protein-bound iodine in the serum of nephrotic patients (3) led Recant and Riggs to reinvestigate this problem (4). They found subnormal concentrations of serum PBI^{127}, normal to high uptakes of I^{131} by the thyroid gland, low basal metabolic rates and high serum cholesterols in their nephrotic subjects. They also noted amounts of PBI^{127} in the urine as high as 36 μg. per day; a decrease in the thyroidal uptake of I^{131} following thyroid therapy; and the ability of the thyroid gland to respond to thyrotropic hormone as measured by a rise in the concentration of serum PBI^{127}. On the basis of these findings, they postulated that in nephrosis the thyroid gland functions normally, that a deficiency of thyrotropic activity is unlikely, that the thyroxine-binding capacity of the plasma proteins is reduced, but that a normal supply of thyroid hormone is delivered to the peripheral tissues (*i.e.*, presumably a normal amount is degraded each day). The low basal metabolic rate and high concentration of serum cholesterol were attributed to some factor other than decreased thyroid function. Although they found protein-bound iodine in the urine, they felt that any significant loss of hormone by this means could be compensated for by increased thyrotropic activity.

Their postulates concerning the abnormality of thyroxine-binding and the normal supply of hormone to the peripheral tissues were made on the basis of indirect evidence. A more direct assessment of these postulates is now possible with the use of thyroxine labeled with I^{131}. The present study was undertaken to determine the abnormalities, if any, of thyroxine metabolism in nephrosis, and to test the validity of the above proposals.

METHODS

One normal subject and three adult patients with the nephrotic syndrome were studied. The diagnosis of the nephrotic syndrome was established in each case on the basis of the presence of gross edema, marked proteinuria, hypoalbuminemia, hypercholesterolemia, a typical electrophoretic pattern of the serum proteins, and the absence of azotemia. The syndrome had been manifest for at least nine months in one patient (A. M.) and for approximately four months in the other two. All three were presumed to be in the nephrotic stage of chronic glomerulo-nephritis.

As part of a previous study (5) each patient had received an intravenous tracer dose of radioactive iodine (I^{131}) three to four weeks before the present study but no residual radioactivity was detectable in serum or urine before the injection of the radio-thyroxine.

From 5 to 15 μg. of L-thyroxine[4] labeled with 50 to 150 μc. I^{131} was administered intravenously to each patient. Plasma samples were obtained at 1, 6, 12 and 24 hours and then daily for 10 days. Twenty-four-hour urine, and three-day stool collections were obtained for 12 days. Duplicate 2-ml. aliquots of each urine and serum sample were prepared. The one was analyzed for total radioactivity and the other for protein-bound radioactivity. The latter was prepared by a modification of the method of Freedberg, Ureles, and Hertz (6), initial precipitation being accomplished with a 10 per cent solution of trichloracetic acid (TCA) and three washes with a 5 per cent solution of TCA. Two-ml. aliquots of the homogenized 3-day stool collections were analyzed for total radioactivity. Radioactivity in all samples was determined by means of a well-type scintillation crystal

[1] Supported by grant AT(30-1)667 from the Atomic Energy Commission.

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[3] Burroughs and Wellcome tablets of desiccated thyroid were used in these studies. Subsequent experience with this material in this laboratory has shown it to be of low and variable potency.

[4] Obtained from Abbott Laboratories, Chicago, Illinois.

TABLE I

Summary of observed and derived values *

Patient		W. L.	L. C.	W. H.	A. M.	Normal values
Diagnosis		Normal	Nephrosis	Nephrosis	Nephrosis	
PBI μg. I/L		60	18	16	19	35–75
U %		40	75	50	46	20–55
E %		58	26	48	54	80–45
BMR		+10	−22	−6	−33	−10 to +15
$T_{\frac{1}{2}}$ days		7.1	3.0	4.2	4.5	5.6–9.5
K_4 %/d		9.8	23.1	16.5	15.4	8–12
K_5 %/d		1.0	7.0	3.5	2.2	
K_6 %/d		8.8	9.0	8.5	9.9	8.9
K_7 %/d		0	7.1	4.5	3.3	0
ETTI μg. I	A	504	191	212	224	350–500
	B		217	224	254	
S.D. Liters	A	8.4	10.6	13.2	11.8	7–11
	B		12.0	14.0	13.3	
S.D. %/body wt.		12	19.6	18.9	15.5	9–12
$[TI]_T$ μg. I/d	A	49	44	35	35	48–100
	B		50	37	42	
$[TI]_F$ μg. I/d		5	13	8	5	5–8
$[TI]_D$ μg. I/d		44	23	19	28	35–95
$[TI]_R$ μg. I/d		0	14	10	9	0
% $[TI]_T$ lost in urine		0	28	27	24	0

* See text for explanation of symbols.

which had a sensitivity of approximately 10^6 counts per minute per microcurie (cpm per μc.). All counts were expressed as per cent of administered dose.

Three-ml. portions of serum, obtained on the second and seventh days, were extracted at pH 2–3 with butanol, evaporated, and chromatographed uni-dimensionally in butanol-dioxane-ammonia after the method of Gross, Leblond, Franklin, and Quastel (7). Thyroxine, triiodothyronine, and diiodotyrosine were identified by adding carrier and spraying with diazotized sulfanilic acid (7). Location of radioactivity was determined by radioautography. A single urine sample from each patient and a single sample of ascitic fluid from one patient (A. M.) was chromatographed in a similar manner. The radioactivity was located by radioautographs and the various spots were cut out and counted in the well-type scintillation crystal.

Serum samples and the ascitic fluid sample obtained on the third post-injection day were studied by zone electrophoresis in filter paper using the method of Kunkel and Tiselius (8). After staining the paper by the technique of Durrum (9) radioautographs were prepared.

Chemical analysis of iodine in serum was performed by a modification of the method of Barker (10).

Basal metabolic rates were determined in the usual manner except that the values were calculated on basis of ideal rather than actual weight.

ANALYSIS OF DATA

Two methods of analysis were employed to determine the size of the extrathyroidal thyroxine iodine pool (ETTI) and quantity of thyroxine iodine metabolized or turned over each day ($[TI]_T$).

Method A (Table I). The plasma disappearance curves of total and protein-bound radioactivity were plotted semilogarithmically (Figure 1) and analyzed by extrapolation techniques (11, 12). The half-time (T1/2) was estimated graphically. The fraction of the body's ETTI turned

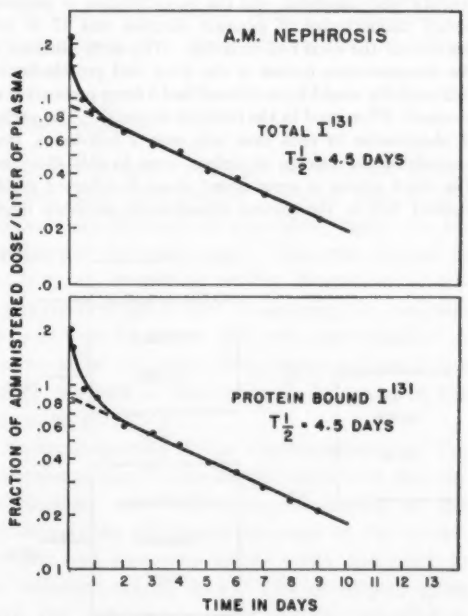

FIG. 1. THE DISAPPEARANCE CURVES OF TOTAL AND PROTEIN-BOUND RADIOACTIVITY IN PLASMA

See text for discussion.

over each day (K_T) was obtained from the known relationship

$$K_T \text{ (Fraction/day)} = \frac{0.693}{T\,1/2} \text{ (days)}$$

The apparent space of distribution (S.D.) of thyroxine was estimated by the extrapolation of the linear curve to zero time.

$$\text{S.D.} = \frac{\text{Total Radioactivity (\%)}}{\text{Conc. Radioactivity (\%/L) at } t_0}$$

This S.D. was also expressed as per cent of body weight.

The ETTI pool was calculated,

$$\text{ETTI } (\mu\text{g. I}) = \text{S.D. (L)} \times \text{PBI}^{127} (\mu\text{g. I/L})$$

and from this the $[TI]_T$ obtained,

$$[TI]_T \text{ } (\mu\text{g. I/day}) = K_T \text{ (fraction/day)} \times \text{ETTI } (\mu\text{g. I})$$

The sources of error in such analytic methods are well known (13) and arise as a result of: 1) The use of non-homogeneous material; 2) failure to observe the disappearance curves for a sufficient period of time; and 3) disproportionate metabolism of the labeled compound during the mixing period (early phase of curves in Figure 1). The first two sources of error were minimized in this study. Material was used which was 95 per cent "pure" chromatographically. This was further checked by measuring both protein-bound and total radioactivity in the plasma. As can be seen in Figure 1, the plasma disappearance curves of the total and protein-bound radioactivity were identical, and the concentration of protein-bound radioactivity of separate samples was 97 to 101 per cent of the total radioactivity. The early portions of the disappearance curves of the total and protein-bound radioactivity would have differed had a large percentage of inorganic I^{131} existed in the injected material. The period of observation in each case was over 2 half-times, thus minimizing the chance of serious error in this direction. The third source of error noted above is inherent in the method, but in the present experiments probably introduced less than a 10 per cent error for S.D. and so did not greatly influence the other values obtained. The values of S.D., ETTI and $[TI]_T$ estimated for the control subject in this experiment, and those obtained for normal subjects by others (11, 12) are in agreement with estimates made by other analytic methods (14). Also the values obtained using biosynthesized organic iodine (15) are similar to those obtained utilizing thyroxine labeled *in vitro* (11, 15), as used in the present experiments.

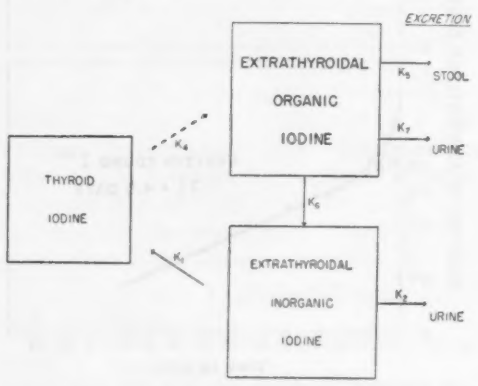

FIG. 2. AN ASSUMED MODEL OF THYROXINE METABOLISM IN NEPHROTIC PATIENTS

See text for explanation.

Method B (Table I). In Figure 2 is represented an assumed model of thyroxine metabolism in the nephrotic subject (modified from Brownell (16)). Radiothyroxine (Tx^*) administered to such an individual enters the extrathyroidal organic iodine pool,[5] from which it leaves either by being excreted in the feces (17) or urine (4) in the form of organic PBI^{131}, or by being degraded in the peripheral tissues with release of inorganic I^{131}. The proportion of Tx^* leaving by each route will depend upon the rate constants K_3, K_6, K_7 which are the fraction of the extrathyroidal organic iodine pool turned over each day by fecal excretion, degradation, and urinary excretion (organic iodine), respectively. The inorganic I^{131} liberated by degradation is either accumulated in the thyroid gland or excreted in the urine, and will be apportioned between the two depending on the rate constants K_1 and K_2 which are the fractions of the extrathyroidal inorganic iodine pool cleared per hour by thyroid and kidney, respectively. Since the rate of removal of I^{131} from the plasma exceeds greatly the rate of degradation of Tx^*, the rate of entry of I^{131} into the urine or thyroid is governed by the rate of Tx^* degradation. As shown by Berson and Yalow (15), the "apparent renal plasma organic iodine clearance" can be estimated. This is the volume of plasma apparently cleared of degraded thyroxine by the kidney each day and is determined as follows,

$$\text{App. Renal PBI}^{131}\text{ Cl. (L/d)} = \frac{\text{Urinary I}^{131}\text{ (\%/d)}}{\text{Mean Plasma PBI}^{131}\text{ (\%/L)}}$$

The urinary inorganic I^{131} was determined by subtracting PBI^{131} from total I^{131}.

Since

$$\frac{K_1}{K_2} = \frac{U}{E}$$

where U is the thyroidal uptake and E the renal excretion of a tracer dose of I^{131}, the "apparent renal clearance" is directly proportional to E, and the "apparent total clearance of organic iodine" by both kidney and thyroid is

$$\text{App. Total PBI}^{131}\text{ Cl.} = \frac{\text{App. Renal PBI}^{131}\text{ Cl.}}{E}$$

Since U and E were determined in each patient shortly before the present studies (5), the above values could be calculated. The amount of thyroxine iodine degraded $[TI]_D$ each day was then estimated,

$$[TI]_D \text{ } (\mu\text{g. I/d}) = \text{App. Total PBI}^{131}\text{ Cl. (L/d)} \times \text{PBI}^{127} (\mu\text{g. I/L})$$

[5] In the ensuing discussion the extrathyroidal thyroxine and organic iodine pools are considered synonymous. This is not strictly true but can be considered so for present purposes.

Determination of the "true renal PBI^{131} clearance" (the volume of plasma cleared of PBI^{131} by the kidney each day) was accomplished similarly,

$$\text{True Renal } PBI^{131} \text{ Cl. (L/d)} = \frac{\text{Urinary } PBI^{131} \text{ (\%/d)}}{\text{Mean Plasma } PBI^{131} \text{ (\%/L)}}$$

and the daily renal excretion of thyroxine iodine $[TI]_R$

$$[TI]_R \text{ (}\mu\text{g. I/d)} = \text{True Renal } PBI^{131} \text{ Cl. (L/d)} \times PBI^{127} \text{ (}\mu\text{g. I/L)}$$

Also the fecal PBI^{131} clearance[6] was determined,

$$\text{Fecal } PBI^{131} \text{ Cl. (L/d)} = \frac{1}{3} \frac{\text{Fecal } I^{131} \text{ (\%/3d)}}{\text{Mean Plasma } PBI^{131} \text{ (\%/L)}}$$

and the daily fecal excretion of thyroxine iodine $[TI]_F$

$$[TI]_F \text{ (}\mu\text{g. I/d)} = \text{Fecal } PBI^{131} \text{ Cl. (L/d)} \times PBI^{127} \text{ (}\mu\text{g. I/L)}$$

Since

$$K_T = K_5 + K_6 + K_7$$

and

$$[TI]_T = [TI]_F + [TI]_D + [TI]_R$$

values for ETTI, S.D., K_5, K_6 and K_7 could be derived,

$$\text{ETTI (}\mu\text{g. I)} = \frac{[TI]_T \text{ (}\mu\text{g. I/d)}}{K_T \text{ (\%/d)}}$$

$$\text{S.D. (L)} = \frac{\text{ETTI (}\mu\text{g. I)}}{PBI^{127} \text{ (}\mu\text{g. I/L)}}$$

$$K_5 = K_T \cdot \frac{[TI]_F}{[TI]_T}$$

$$K_6 = K_T \cdot \frac{[TI]_D}{[TI]_T}$$

$$K_7 = K_T \cdot \frac{[TI]_R}{[TI]_T}$$

Each of the above clearance values was determined in each patient on four separate 24-hour periods and the arithmetical mean obtained. The mean plasma concentrations were determined graphically and checked by the formula

$$P = \frac{P_1 - P_2}{2.3 \log \frac{P_1}{P_2}}$$

which is the expression of the true mean of an exponential dP/dt. The calculated and graphically obtained mean concentrations were, for practical purposes, identical.

Since the estimation of the daily amount of thyroxine iodine degraded $[TI]_D$ involves the use of previously determined U and E, this value may be in error if the true uptake is different from the observed, or if the uptake changed before the present study was initiated. Also, as shown in Figure 2, inorganic I^{131} liberated by degradation and taken up by the thyroid may be synthesized into Tx* and secreted into the circulation. However previous studies with I^{131} in these subjects (5) had shown that the turnover of I^{131} accumulated by the thyroid was slow, and that the PBI^{131} released into the circulation never reached a plasma concentration above .02 per cent per L. Such values would introduce an error of 2 to 3 per cent or less.

A final assumption was that these patients were in metabolic equilibrium as regards thyroxine secretion and turnover so that $[TI]_T$ was assumed to equal the daily secretion of thyroxine iodine by the thyroid gland.

[6] The nature of the fecal I^{131} was not determined. In the calculations and in the subsequent discussion it has been assumed that the thyroxine iodine is cleared by the gastrointestinal tract in an organic form.

RESULTS

Electrophoresis

Zone electrophoretic analysis of the serum from each patient revealed similar patterns in all instances. There was a high $alpha_2$-globulin and low albumin. All the radioactivity detected by radioautography appeared in a single band having a mobility intermediate between that of $alpha_1$- and $alpha_2$-globulins. These results coincide with those obtained by similar analyses of normal serum after either intravenous administration of labeled thyroxine, the addition of labeled thyroxine *in vitro*, or the biosynthesis of labeled hormone (18–20).

A similar analysis of the ascitic fluid (patient A. M.) gave comparable results. The radioactivity appeared as a single band in the inter-alpha zone.

Chromatography

Chromatographic analysis of the two serum samples from each patient revealed that all the radioactivity detected by radioautography was located in the thyroxine spot. This was checked in one instance by cutting out the thyroxine and triiodothyronine spots, and measuring the radioactivity in each by means of a well-type scintillation crystal. The triiodothyronine spots contained less than 1 per cent of the radioactivity found in the thyroxine spots.

Radioautographs of the chromatograms of the butanol-extracted urine samples indicated that the radioactivity was located predominantly in the thyroxine spot with lesser amounts in the diiodotyrosine and inorganic iodide spots and none in the triiodothyronine spot. The developed spots were cut out and counted in the scintillation counter. The urinary diiodotyrosine was found to represent 6 to 8 per cent of the butanol-extractable organic iodine. This is comparable to values

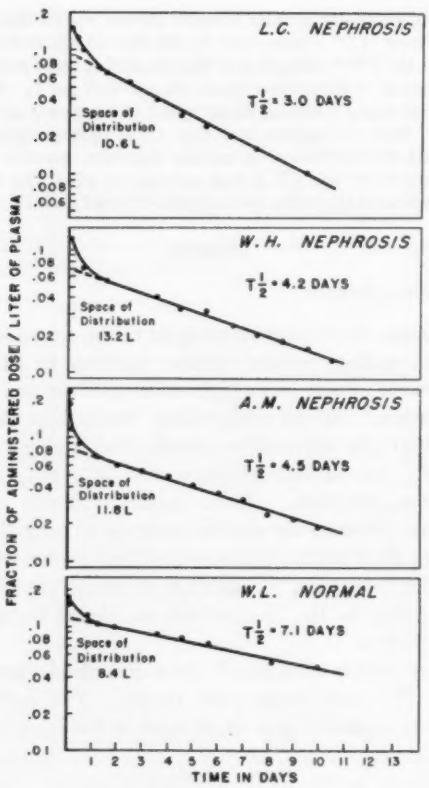

FIG. 3. DISAPPEARANCE CURVES OF PROTEIN-BOUND RADIOACTIVITY IN THE PLASMA OF THREE NEPHROTIC SUBJECTS AND ONE NORMAL SUBJECT

reported for normals (21). A similar analysis of the supernatant after the precipitation of the urine with TCA revealed only inorganic I^{131}. Therefore, the labeled diiodotyrosine was included as urinary PBI^{131} in the calculations of Method B. If this labeled diiodotyrosine came from the breakdown of thyroxine filtered through the renal glomeruli, no appreciable error would be introduced in the calculations of the "true" and "apparent" renal clearances of PBI^{131}. On the other hand, the estimate of "true" renal clearance of PBI^{131} would be high and that of "apparent" renal clearance of PBI^{131} low, if the diiodotyrosine resulted from the degradation of thyroxine which had reached the renal cell from the post-glomerular blood stream.

When the single sample of ascitic fluid was analyzed as above, the radioactivity was located entirely in the thyroxine spot.

Serum and urine radioactivity

The plasma disappearance curves of PBI^{131} for the normal and three nephrotic subjects are shown in Figure 3. The $T\frac{1}{2}$ for the normal subject was 7.1 days, which is within the normal range (5.2 to 9.5 days; mean 6.5 days), as reported by others (11, 14, 15). The $T\frac{1}{2}$ in each nephrotic patient was less than normal (3.0 to 4.5 days).

The curves of appearance of both total and protein-bound radioactivity in the urine of patient W. H. are shown in Figure 4. As expected, they resemble closely the plasma disappearance curves and have similar $T\frac{1}{2}$'s. As can be seen, the protein-bound I^{131} was a constant proportion of the total I^{131} excreted. Thus the previous assumptions, on which the calculations of Method B are based, appear valid.

Derived values

Table I lists the observed and derived values for each patient obtained by both methods of analysis.

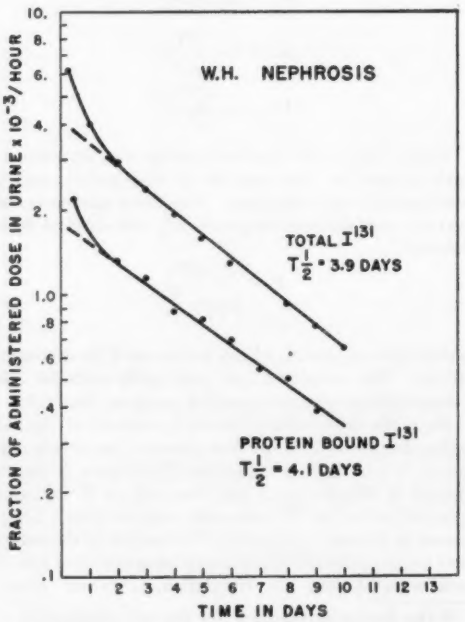

FIG. 4. APPEARANCE CURVES OF TOTAL AND PROTEIN-BOUND RADIOACTIVITY IN THE URINE OF A NEPHROTIC SUBJECT

Also listed are the values for the normal subject, and the average normal values obtained by others (11, 14, 15). In the nephrotic patients the ETTI (191 to 224 μg. I) was less than normal (350 to 500 μg. I) and the apparent space of distribution of thyroxine (10.6 to 13.2 L.) was greater than normal (8 to 11 L.), particularly when expressed as per cent of body weight (nephrotics 15.5 to 19.6 per cent; normals 9 to 12 per cent). The daily turnover and synthesis of thyroxine iodine $[TI]_T$ was normal or only slightly decreased (nephrotics 35 to 44 μg. I/d; normal 38 to 100 μg. I/d), but owing to the fact that there is a significant daily loss of organic iodine in the urine (9 to 14 μg. I), and an increased proportion of iodine lost in the stool (nephrotics 12 to 26 per cent; normal 10 to 12 per cent), the amount of hormonal iodine degraded each day (19 to 28 μg. I) is significantly less than normal (35 to 90 μg. I) and falls in the range reported for patients with panhypopituitarism (16.6 to 29.8 μg. I) (14). The percentage of ETTI turned over each day by degradation, K_d, is normal. This signifies that these nephrotic patients turned over thyroxine iodine at a more rapid rate than normal owing to the loss of organic iodine in the urine and a proportionately increased loss of iodine in the feces, but not because of an increased rate of peripheral degradation as found in thyrotoxic individuals (14, 15).

The values for ETTI, S.D. and $[TI]_T$ obtained by the two methods are approximately equal.

DISCUSSION

If, as proposed by Recant and Riggs (4), there is an abnormality in thyroxine-binding, the concentration of PBI^{127} in the serum would be low as seen; but the ETTI would be normal not low; the apparent space of distribution of thyroxine (S.D.) a great deal larger than that actually found; and the early phase of the plasma disappearance curves of radioactivity would have fallen more rapidly and to a lower level than observed in the present study. Thus the present data do not support their above-mentioned concept, but suggest that the low PBI^{127} in the serum is the result of other causes. This agrees with the conclusion of Robbins, Rall, and Petermann (22). These authors studied the thyroxine-binding capacity of nephrotic serum *in vitro* and found it to be slightly reduced but felt that this reduction was not sufficient to account for the lowered concentration of PBI^{127} in the serum.

Thyroid function as estimated in the present studies was "normal," in the sense that the uptake of inorganic I^{131} by the thyroid was in the normal range. Also the estimated daily secretion of thyroxine by the thyroid gland in each case approximated or was slightly less than that found in normal subjects. However, the amount of hormone undergoing peripheral degradation each day was estimated to be approximately one-half the normal amount. This apparent discrepancy between the quantity of thyroxine secreted by the thyroid and that degraded in the peripheral tissues is explained by the finding of a significant loss of thyroxine in the urine (25 to 30 per cent of that secreted) and a proportionately increased loss of thyroxine or its degradation products in the feces. If the amount of thyroxine degraded peripherally is directly related to the metabolic rate then these patients can be considered to have "tissue hypothyroidism" (*i.e.*, less than the normal supply of thyroxine reaches and is degraded by their peripheral tissues). This might at least partially explain the hypometabolism observed in such patients.

The subnormal concentration of PBI^{127} in the plasma of nephrotic individuals should result in an increased production of thyroid stimulating hormone (TSH) by the anterior pituitary gland, if as postulated (23), the plasma level of thyroid hormone is a significant factor in regulating the release of TSH from the pituitary. In turn, the increased TSH should stimulate the thyroid in such a way as to cause both an increased rate of uptake of inorganic iodine and an increased rate of thyroxine secretion. In spite of the low concentration of PBI^{127} in their serum, the thyroid glands of the present patients apparently are not secreting increased amounts of thyroxine. Presumably this could result from a functional abnormality in either the thyroid or anterior pituitary gland. By the administration of TSH, Recant and Riggs (4) were able to cause a rise in the concentration of PBI^{127} in the serum of two nephrotic subjects. This would suggest that the postulated defect lies in the pituitary gland, but does not exclude the possibility that the thyroid gland or the mechanism controlling thyroxine release is less sensitive to a physiological amount of TSH.

The finding of a normal or high thyroidal uptake of I^{131} would appear to be inconsistent with the proposed pituitary defect. Several factors may explain this apparent inconsistency. In the first place, it is obvious that there is not an absolute lack of TSH activity since the rate of hormonal secretion is maintained at nearly normal levels. Secondly, although the uptake of I^{131} is normal or high, the thyroidal clearance of I^{131} (K_1) appears normal (3.0 to 5.0 per cent per hr.) (5). This is in contrast to values found in states of supposed increased anterior pituitary activity such as iodine deficiency or thyrotoxicosis (8 to 12 per cent per hr.). In other words, the normal or high uptake of I^{131} results not from an increased thyroidal clearance (K_1), but from a decreased renal clearance of I^{131} (K_2). Thirdly, it has been shown (24) that the iodine accumulating function of the thyroid gland is at least partially independent of anterior pituitary stimulation, varying with the iodine content of the diet in hypophysectomized animals. The reason for this presumed lack of augmented TSH output is not clear, but in these patients who are chronically ill and possibly protein-depleted a disturbance in pituitary function similar to that seen in chronic human malnutrition (25) seems possible.

As indicated by Recant and Riggs (4) and confirmed by Cruchaud, Mahaim, Scazziga, and Vannotti (26), some patients with this syndrome have abnormally high thyroidal I^{131} accumulation gradients or conversion indices in contradistinction to the normal thyroidal clearances observed in the present subjects (these three measurements, although expressed differently, measure the same parameter of thyroid function). It is possible that in such individuals the pituitary-thyroid axis is able to respond to the increased loss of thyroxine and thereby attempt to maintain an euthyroid state. This may also explain why some patients with this syndrome have normal concentration of protein-bound iodine in their serum. The authors wish to emphasize the point that all the subjects of the present investigation were adults whose disease was chronic and in whom the measureable indices of the disease (body weight, degree of albuminuria, serum concentration of albumin and cholesterol, etc.) were nearly constant during the period of study. It is not surprising, therefore, that they formed a fairly uniform group as regards their metabolism of thyroxine. However, they were observed for only a brief period during the long evolution of their disease. The results of similar studies in other individuals with the nephrotic syndrome may well differ from those recorded herein, since it is more than likely that as the syndrome evolves progressive alterations in thyroid function occur. If this is the case, then the data obtained in studies of this nature will depend upon when, during the course of the disease, such studies are carried out. In other words, the authors suggest that the variations in thyroidal function occurring in the nephrotic syndrome are dynamic in nature and evolve as the syndrome evolves, and that only by further work will it be possible to completely define these sequential changes.

The results of the present investigation suggest that the low concentration of PBI^{127} in the serum of some patients with the nephrotic syndrome is a result of at least four factors:

1) a significant loss of organic iodine in the urine;
2) a proportionately greater loss of iodine in the feces;
3) dilution of the organic iodine present because of an expanded extracellular fluid volume, and
4) inability of the pituitary-thyroid axis to augment the output of thyroxine in the face of an apparent thyroxine deficiency.

SUMMARY

The metabolic fate of I^{131}-labeled thyroxine in three patients with the nephrotic syndrome is described, and the following conclusions drawn:

1. The half-time of plasma disappearance ($T\frac{1}{2}$) of intravenously administered I^{131}-labeled thyroxine is shorter than normal.

2. There is a significant loss of organic iodine in the urine, and a proportionately increased loss of iodine in the feces.

3. The organic iodine in the urine is primarily in the form of thyroxine, but a small percentage (6 to 8 per cent) exists as diiodotyrosine.

4. The transport of thyroxine by the plasma proteins appears normal.

5. The amount of hormone degraded by the peripheral tissues is in the hypothyroid range, and this finding may explain in part the lowered basal metabolic rate seen in these patients.

6. There is an apparent defect in the response of the pituitary-thyroid axis to the lowered concentration of PBI^{127} in the serum.

The observations are discussed in the light of previous studies on iodine metabolism and thyroid function in nephrosis.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Dr. John Stanbury for supporting this work and for many helpful suggestions, and to Dr. Gordon Brownell for his assistance in analysis of the data. Also, one of the authors (H. R.) wishes to thank Dr. Walter Bauer for his help and encouragement.

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PHYSICOCHEMICAL ESTIMATION OF ALDOSTERONE IN URINE[1]

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(Submitted for publication December 27, 1955; accepted March 28, 1956)

We have recently described (2) the essential features of a physicochemical method for the specific determination of aldosterone in body fluids and tissues. This method has become of special interest to clinical groups, particularly for the determination in urine. In the meantime, it has been possible to improve and simplify the method over several hundred determinations, so that it is now suitable, as a standardized procedure, for use in routine clinical investigations of urine. These reasons prompt us to publish the following modified and detailed description, so that the delicate procedure should be perfectly reproducible.

The method consists of three steps: 1. Hydrolysis and extraction of the urine, 2. paper chromatography of the extracts with the help of two solvent systems, in which the relative running rates of aldosterone can be differentiated from those of other corticosteroids, and 3. semiquantitative estimation of the aldosterone.

MATERIALS

Methanol: distilled; Ethyl acetate: distilled.

Acetone: shaken with calcium chloride, filtered and distilled.

Chloroform: washed with water, shaken with calcium chloride, filtered and distilled.

Toluene: emulsified three times with 0.07 vol. of conc. sulphuric acid at room temperature in a Vibro-mixer for five minutes, separated, washed 5 times with 0.2 vol. of water to neutral, dried over phosphorus pentoxide and distilled *in vacuo*.

Light petroleum: distilled, fraction with b.p. 50–70° C.

Formamide: Merck, chromatographic grade.

Whatman paper No. 1 (for preparative chromatogram): extracted in a Soxhlet apparatus for 2 days with 95 per cent methanol and subsequently for 2 days with chloroform.

Sudan III: saturated solution in hexane.

Alkaline blue tetrazolium solution: mixture of 10 parts 0.1 per cent aqueous solution of blue tetrazolium (B.D.H., London) and 90 parts 2 N sodium hydroxide solution; may be stored for 1 to 2 days.

Reference substances: The solutions of cortisone and hydrocortisone in methanol should be stored (for not longer than 2 weeks) in small tubes with a double stopper, only the outer stopper being greased. Aldosterone solutions are unnecessary.

METHOD

1. Urine sample

The urine is cooled and collected over a 24-hour period with addition of about 10 ml. chloroform. If working up is not possible at once, the urine is frozen and stored at about − 10° C. In general, at least half of the urine collected during 24 hours should be used; if a high aldosterone content is expected, correspondingly smaller amounts will suffice.

2. Hydrolysis and extraction

The urine is acidified to pH 1.5 with conc. HCl at 10 to 15° C. After standing for 24 hours at 15 to 20° C., the extraction is carried out in a separating funnel fitted with a stirrer.

Thus, 1 vol. urine is stirred 4 times with 0.2 vol. chloroform for 15 to 20 minutes at room temperature, in such a way that good mixing of the two phases occurs without formation of emulsions separable only by centrifuging. The combined chloroform solutions (= 1 vol.) are washed first with 0.05 vol. cold 0.1 N sodium hydroxide solution, then three times with 0.05 vol. water. The washings are twice back extracted with 0.05 vol. chloroform. All the chloroform solutions are now combined, dried quickly over a small amount of sodium sulphate, filtered, and evaporated *in vacuo* not over 50° C. If a capillary-leak is used, this should be with nitrogen. The residue is dissolved in a little acetone, the solution filtered through a small cotton-wool pad into a weighed test tube and again evaporated *in vacuo*. This solution is dried at 60° C. for about ½ hour *in vacuo* and weighed.

On average, these extracts of 12 to 24-hour

[1] Communication No. 138 "On Steroids." No. 137 compare (1).

urine specimens weigh 10 to 60 mg., and are suitable for preparative paper chromatography without further purification. Should the extracts in special cases exceed this amount, they must be first chromatographed on silica-gel, as described earlier (2).

3. *Preparative paper chromatography*

One to five sheets of washed Whatman paper No. 1 (see Figure 1) are drawn through a 30 per cent solution of formamide in acetone, and allowed to hang for 5 minutes from the upper edge. After this time, the impregnated paper is ready to receive the chloroform extract. About 10 to 12 mg. of this is dissolved in 0.1 ml. methanol-chloroform (1 : 1), and distributed in a line on each sheet, by moving the micropipette backwards and forwards evenly over the start line, keeping the outflow rate as constant as possible. The test tube is rinsed three times with 0.02 ml. solvent, and this solution also distributed over the sheets. A space of 3.5 cm. is left free in the midle of one of the sheets where the reference substances, 10 μg. of cortisone and hydrocortisone, respectively, in 0.2 per cent methanol solution, are dropped on. Two to five μl. of a solution of Sudan III may be dropped on one end of the start line, this indicator moving with the solvent front. The chromatograms are developed by the descending technique at a constant temperature (*e.g.*, 20 to 22° C.) using chloroform saturated with formamide (3). Paper chromatographic practice must be assumed here, and will not be described further. See *e.g.* the general reviews of Cramer (4), and of Block, Durrum, and Zweig (5), the papers of Zaffaroni (3) and Bush (6), and the references given therein. By the procedure outlined above, the solvent front reaches the bottom in 2.5 to 3 hours. The sheets are carefully removed from the trough and allowed to dry, preferably overnight, at room temperature and protected from light.

4. *U.V. photocopying and evaluation*

The chromatograms are notched on one side, spread over photographic paper in the dark room (Markham and Smith [7]), and illuminated at a distance of 35 to 40 cm. by a U.V. lamp for 4 to 5 seconds, with the aid of a shutter on the underside of the lamp housing (light source: GE Germicidal Lamp, 8 watt, without filter). The exposed photographic papers are developed, fixed, washed and dried in the usual way.

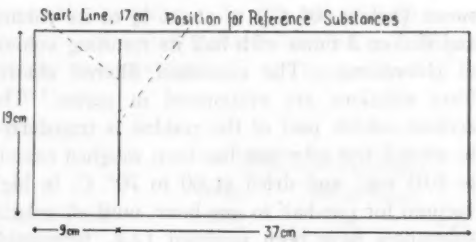

FIG. 1. DIAGRAM OF PAPER SHEET USED IN CHROMATOGRAPHY

In the formamide/chloroform system, aldosterone travels almost as far as cortisone. A line is therefore drawn across the reference photo-copy at the lower limit of the cortisone spot, and a parallel one about 5 cm. above it. The location of cortisone and aldosterone on the photo-copies of the chromatograms without the reference substances can easily be established by comparison with the first sheet. For this purpose the pattern on the photo-copy formed by the accompanying U.V.-absorbing material is very useful. For example, there is often a strongly absorbing band just ahead of the cortisone, but which has nothing to do with either cortisone or aldosterone. The chromatograms are now lined up with the corresponding photo-copy, and the two lines traced on the chromatograms. The bands thus marked, containing as hormones only cortisone and aldosterone, are cut out. The cortisone chromatographed jointly as a reference substance does not affect the determination since it is separated, together with the urine cortisone, from the aldosterone in the following chromatogram.

5. *Elution of the paper strips containing aldosterone*

a) The strips removed are cut up and shaken with sufficient 20 per cent methanol in a glass-stoppered round-bottomed flask to obtain a thick pulp. After standing for half an hour at room temperature, the solution is sucked through a sintered glass filter into another flask and the pulp residue *slowly* washed twice with methanol and pressed out. The filtrate is concentrated *in*

vacuo (below 60° C.) to about ⅛ of its volume and shaken 3 times with half its resulting volume of chloroform. The combined filtered chloroform solutions are evaporated *in vacuo*. The acetone-soluble part of the residue is transferred to a small test tube that has been weighed exactly to 0.05 mg., and dried at 60 to 70° C. in high vacuum for one-half to one hour, until all volatile substances have been removed (*e.g.*, formamide from the chromatography), then weighed. At this stage the extracts weigh about 0.5 to 4 mg. per 12 to 24-hour urine volume.

b) A somewhat simpler elution procedure may be used just as effectively. The paper strips cut-out are attached singly in 5 cm. broad syphons [2] made of aluminum sheet and packed with glass wool as shown in Figure 2a. The syphon is put into a shallow dish filled with 80 per cent methanol. Before the solvent front has reached the lower edge of the paper strip, a three-cornered piece of filter paper (Whatman No. 1), saturated with 80 per cent methanol, is hung on this to facilitate the draining of the solvent (Figure 2b). For complete elution, at least 15 ml. of solvent is allowed to run off for at least half an hour into a small round-bottomed flask, the whole being protected from draughts. The eluate is then evaporated *in vacuo* and taken up in a small quantity of acetone, the soluble part being filtered through a small cotton-wool pad into a weighed test-tube and treated as described in a). In recovery experiments it was found that by both methods about 90 and 95 per cent, respectively, out of 5 and 20 μg. aldosterone on the paper could be eluted. When it is necessary to elute several strips together, method a) is preferable. The latter also gives purer eluates, which may be advantageous in the second chromatography.

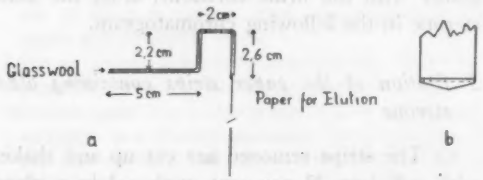

FIG. 2. DIAGRAM OF ALUMINUM SYPHON USED FOR ELUTION

a—Side view.

b—Three-cornered paper tab saturated with 80 per cent methanol attached to the end of the strip to facilitate draining of the solvent.

[2] Suggested by Miss M. L. Helmreich, Salt Lake City.

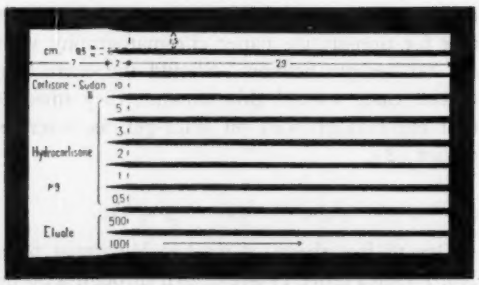

FIG. 3. DIAGRAM OF ARRANGEMENT OF PAPER STRIPS EMPLOYED IN THE SEPARATION OF ALDOSTERONE BY THE SECOND PAPER CHROMATOGRAPHY

6. *Separation and estimation of aldosterone by the second paper chromatography*

From the extracts obtained above, 100 μg. and 500 μg., and also—if the amount of extract permits or the low aldosterone content necessitates—1,000 μg., are run by descending technique in Bush's system C (6) parallel with 0.5, 1, 2, 3, and 5 μg. of hydrocortisone and 10 μg. cortisone.

Example. 1.7 mg. Extract (*i.e.*, the eluate from the first chromatogram) was dissolved in 85 μl. methanol-chloroform (1:1) and 5 and 25 μl., respectively (corresponding to 100 and 500 μg. substance) were dropped on the start line of two adjacent paper strips (Whatman No. 1, dimensions and setting out as in Figure 3). On similar paper strips 5, 10, 2 × 10, and 3 × 10 μl. of a 0.01 per cent methanolic solution of hydrocortisone (corresponding to 0.5, 1, 2, and 3 μg.) were dropped, as well as 10 μl. of a 0.05 per cent solution of hydrocortisone (corresponding to 5 μg.) and 10 μl. of a 0.1 per cent solution of cortisone. The strips were hung immediately in the dry trough of the chromatography tank, well saturated with the phases of Bush's solvent system C, and equilibrated at the temperature at which the chromatogram is to be run (preferably 40° C.). The solvent was then run into the trough. After the solvent front had reached the lower edge of the paper (about 2 hrs., observed by means of a little Sudan dye running simultaneously), the strips were removed, and allowed to dry for about an hour in air away from light.

For the evaluation of the chromatogram two combined color reactions are used, one depending on the reducing α-ketol side chain, the other on the Δ^4-3-ketone group in aldosterone. The strips are quickly dipped in an alkaline solution of blue tetra-

zolium contained in a well-filled shallow dish, then with the aid of a glass rod laid directly on a white opaque glass plate and evaluated within 5 minutes. In a very short time, blue to violet spots appear wherever there are reducing compounds. Aldosterone separates readily from cortisone in Bush system C, but runs at practically the same rate as hydrocortisone from which it has, of course, already been separated in the preparative chromatogram. Therefore, a blue spot in the same position as hydrocortisone on the second chromatogram indicates, together with the sodium hydroxide fluorescence (see below), the presence of aldosterone. In the case of normal urine, the aldosterone content is so small (about 1 to 9 μg. per 24 hours) that usually a weak spot appears only in the 500 μg. chromatogram, while in hyperaldosteronuria, distinct spots may be seen in the 100 μg. chromatogram. The sensitivity limit of the blue tetrazolium reaction of aldosterone is about 0.2 μg. per cm.^{2} of the chromatogram. The aldosterone spots, located above the cortisone spots, are visually correlated against the hydrocortisone spots. (The slight differences between the sensitivities of aldosterone, cortisone, and hydrocortisone are insignificant for this semiquantitative estimation.)

After one-half to one hour, the strips are rapidly dried, hanging for 15 to 20 minutes in a drying oven at 90° C. in a weak draught, and then observed in U.V. light from a mercury vapor lamp fitted with a filter (max. radiation at 300 to 370 mμ). In place of the blue formazan spots, a yellow fluorescence is seen wherever Δ^4-3-ketosteroids are present. The spots drift somewhat during the drying process, which does not, however, affect the visual estimation under the U.V. lamp. The sensitivity of this soda-fluorescence reaction depends on the drying conditions and is optimally about 0.1 μg. per cm.^{2} of the chromatogram. The same sensitivity can be obtained by an appropriate drying procedure with an infrared lamp. The variation between this estimation and that obtained with blue tetrazolium should not be more than 50 per cent for low concentrations, and not more than 30 per cent for higher concentrations. If there is a comparatively large discrepancy, the lower value only should be taken, otherwise the mean of the two determinations. Normally the values found by the soda fluorescence are somewhat lower than those by the blue tetrazolium reaction. By the use of *both* tests, the standard deviation can be kept below ± 20 per cent for concentrations of 1 to 5 μg. per spot (2 to 3 cm.^{2}).

Example

	Aldosterone evaluation from the chromatograms containing		
	100 μg.	500 μg. extract	
Reduction test	0.75 μg.	4 μg.	= 0.8%
NaOH test	0.5 μg.	3.5 μg.	= 0.7%
Mean value			= 0.75%

In this case the smallest margin of error was expected from the 500 μg. chromatograms, so the mean value from these alone was used in the estimation. For 1.7 mg. eluate from 900 ml. urine, this corresponds to 12.8 μg. per 900 ml., and with 1,150 ml. per 24 hours, to 15.4 μg. per 24 hours, or in round figures, to 15 μg. per 24 hours.

DISCUSSION

a) *Hydrolysis and extraction*

The aldosterone excreted with the urine is present to a large extent in a bound state (2). On the one hand the hydrolysis at pH 1 for 24 hours (method d) has been shown (2) to release more aldosterone than that at pH 1 for only about 30 to 60 minutes (method c) or with β-glucuronidase (method b). On the other hand, free aldosterone is not completely stable at pH 1 over 24 hours (8). A small amount of aldosterone released by method d may therefore be assumed to be destroyed. A comparison of method c and d, whereby free aldosterone was added to urine, showed that with method d, 10 to 15 per cent aldosterone is lost. Nevertheless, using a double extraction, first by method c when practically no aldosterone is destroyed (8, 9), then by method d, the recovery of relatively small amounts of aldosterone excreted with the urine was hardly any better than with method d alone. The main bulk of the aldosterone is, in fact, not extractable under the inadequate hydrolytic conditions of method c, but is liberated only in method d, and therefore still exposed to the 24-hour-action of the acid. Moreover, it seems likely that the bound form of aldosterone is less affected by the acid than the free hormone. Finally, the time-consuming double extraction yields a larger amount of inert material which requires more paper sheets for the preparative chromatography, thereby causing additional losses (see below). For these reasons we use only method d.

b) *Chromatography*

The losses in the preparative paper chromatography in formamide/chloroform (including the elution) were investigated. Samples of 2 and 20 μg. pure aldosterone added to 10 mg. each of an aldosterone-free extract prepared by method d from the urine of a patient with Addison's disease were chromatographed and eluted. On average, 83 and 92 per cent, respectively, of aldosterone were recovered. The losses caused by both chromatography and elution are thus more important for low aldosterone concentrations than for higher ones. This result implies that the loss also depends on the number of sheets used. It is therefore advantageous in a series of experiments always to use the same number of sheets for the preparative chromatography, *i.e.*, as far as possible the same urine equivalent per determination.

c) *Total yield and reproducibility.*

Five, 10 and 20 μg. aldosterone, respectively, were added to 500-ml. amounts of normal urine. The samples were frozen for 3 to 7 days, then thawed and worked up by the present method, two sheets of paper being used for each determination (quantity of extract 12.2 to 22.6 mg.). After subtracting the aldosterone originally present in the urine, averages of 68, 76 and 86 per cent, respectively, of aldosterone were recovered depending on the amount added, the average thus being 75 per cent for this order of concentration. It should be mentioned that seemingly small variations of method can in some cases diminish the yield considerably. The reproducibility of the determinations under carefully standardized conditions was satisfactory, the standard deviation being less than ± 20 per cent. If the amounts of aldosterone were very small, or when separate estimations were carried out by different operators, the standard deviation was as high as ± 25 to 30 per cent.

The aldosterone extractable by the present method is estimated accurately enough for clinical purposes. However, the total aldosterone content in the urine remains somewhat uncertain, since the type of conjugation of aldosterone in the urine, and the absolute effectiveness of the hydrolysis is not sufficiently known.

d) *Results*

In comparison with the biological test methods, we have obtained slightly higher values. We ascribe this particularly to the possibility that in biological testing of the extracts, even when chromatographed, unknown factors with antagonizing activity are involved (10). In some cases this assumption could be confirmed, as we were able, despite low biological results, to isolate a high amount of crystalline aldosterone that corresponded to that found in the physicochemical test (1, 11, 12). Moreover, Llaurado's very low recovery value of 20 per cent of the added aldosterone (13), determined biologically, could be eventually explained by such antagonistic substances. Finally it must be emphasized that we consider the use of two different solvent systems essential for the complete separation of the aldosterone.

The aldosterone excreted by healthy adults and determined by our method lies mostly between 1 to 9 μg. per 24 hours (14), or 0.5 to 6.5 μg. per 24 hours (15), as against 1.5 to 5 μg. (16) and 1 to 3 μg. (17) found in biological tests and with slightly different extraction and chromatography techniques. In 52 estimations in 16 persons who were clinically quite normal the range was 0.5 to 12.5 μg. per 24 hours ($P = 0.05$). In this connection it must be remembered that aldosterone excretion is closely related to the water, sodium and potassium intake, as well as to the renal and extrarenal water excretion (18–20). If these are not defined beforehand, it will be difficult to make a clear distinction between normal and slightly raised values.

The results obtained with this method in various illnesses (14), for instance in congenital adrenogenital syndrome (10), in cirrhosis of the liver (21, 15), in hyperaldosteronuria, produced by a potassium load or by perspiration (18), and in postoperative patients (22), have been reported in part elsewhere.

SUMMARY

A detailed description of a specific, physicochemical method for the determination of aldosterone in the urine is given. Errors due to loss and variation are discussed, as well as the possible differences between the results using our method

and those using biological test methods. Values for urines of normal adults are given.

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RADIOTHYROXINE TURNOVER STUDIES IN MYXEDEMA, THYROTOXICOSIS, AND HYPERMETABOLISM WITHOUT ENDOCRINE DISEASE

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(Submitted for publication February 8, 1956; accepted March 15, 1956)

In a previous report (1) the disappearance curves of I^{131}-labeled L-thyroxine and L-triiodothyronine were described in euthyroid subjects. The radioactive compounds were injected intravenously, and the radioactivity of the plasma or serum was determined at intervals for approximately two weeks. Estimations were made of the rate of degradation of organic (thyroxine) iodine. In the present work, this approach has been extended to myxedematous and thyrotoxic patients with findings agreeing in general with Ingbar and Freinkel (2) whose methods were similar, as well as Berson and Yalow (3) who employed entirely different methods. In the effort to elucidate the role of peripheral tissue metabolism in hormone degradation rate, studies were carried out during treatment of myxedema, and in subjects exhibiting hypermetabolism without evidence of endocrine disease.

MATERIAL AND METHODS

Tracer amounts of I^{131}-labeled L-thyroxine [1] were injected intravenously, and heparinized venous blood samples were taken 10 minutes after injection and daily or on alternate days for two weeks or longer. Prior to injection the radiothyroxine was kept in siliconed glassware and syringes to prevent adsorption by vessel walls. The plasma radioactivity was assayed in a well-type scintillation counter which recorded approximately one million counts per minute per microcurie of I^{131} (42 per cent overall efficiency) above a background of 130 cpm. Corrections for radioactive decay were made when necessary. Usually all plasma samples from a given patient and diluted aliquots of the administered compound were counted together after conclusion of the study, obviating the need for decay correction.

In all subjects one or more plasma protein bound iodine (PBI) or butanol extractable iodine (BEI) determinations were carried out [2] (4, 5). The BEI was done to avoid interference from inorganic iodine previously administered in the three instances where this had occurred. The two determinations were used interchangeably in the calculations of "organic iodine" figures; PBI ordinarily exceeds BEI by approximately 0.6 μg. per cent in the absence of inorganic iodine (5).

Calculations

The injection of radiothyroxine was followed by a relatively rapid initial fall in radioactivity of the plasma (Figure 1). This was attributed to diffusion of the tracer out of the vascular compartment as it was distributed throughout the body's extrathyroidal organic iodine (EOI) pool. After two days the plasma radioactivity declined more gradually, resulting in a straight line when plotted semilogarithmically. This slow exponential component of the disappearance curve was interpreted as

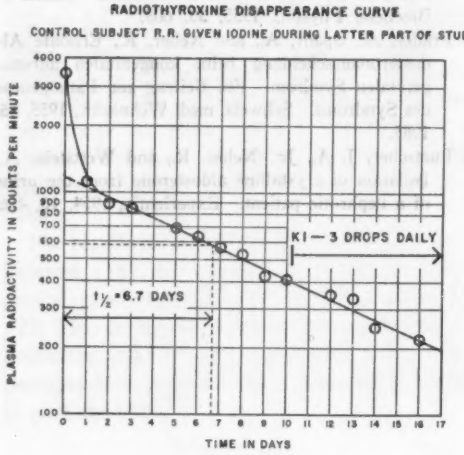

FIGURE 1

Semilogarithmic plot.

[1] Approximately 50 μc. in 2 to 10 μg. thyroxine, supplied by Abbott Laboratories, Oak Ridge, Tennessee.

[2] Performed by Bio-Science Laboratories, 2231 South Carmelina Avenue, Los Angeles 64, California and in some instances by Dr. Belton A. Burrows, Veterans Administration Hospital, Boston, Mass.

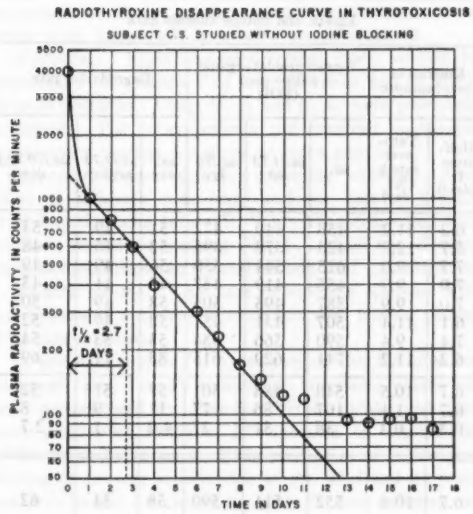
RADIOTHYROXINE DISAPPEARANCE CURVE IN THYROTOXICOSIS
SUBJECT C.S. STUDIED WITHOUT IODINE BLOCKING
PLASMA RADIOACTIVITY IN COUNTS PER MINUTE
TIME IN DAYS
t½ = 2.7 DAYS

FIGURE 2

Semilogarithmic plot. The flattening of the curve after the eighth day was attributed to reutilization of the I^{131} label from degraded radiothyroxine and its reappearance in the circulation as newly synthesized radiothyroxine. With iodine prefeeding, the curves remained linear throughout in thyrotoxic patients.

metabolic degradation of the administered radiothyroxine, hence a measure of the turnover rate of the hormone.

The half-time of thyroxine turnover was obtained graphically from the linear component of the disappearance curve extrapolated back to zero time (Figure 1). The turnover rate was computed from the half-time.[8]

As an example, the calculations for subject R. R. follow:

Half-time ($t_{\frac{1}{2}}$) = 6.7 days (obtained graphically. Figure 1).

$$\text{Turnover rate (k)} = \frac{\ln 2}{t_{\frac{1}{2}}} = \frac{0.693}{6.7} = 0.104$$

$$= 10.4\% \text{ per day.}$$

This turnover rate indicated the fraction of the body's extrathyroidal organic iodine (EOI) pool synthesized and degraded daily. The EOI pool was estimated from the quotient

$$\frac{\text{Total radioactivity injected}}{\text{Radioactivity per microgram of PBI}}.$$

The denominator was obtained from the zero time extrapolation which represented plasma radioactivity if distribution had occurred instantaneously after injection.

Plasma PBI = 5.8 μg. per 100 ml.
Zero time plasma radioactivity = 1,150 cpm per ml.

$$\frac{1{,}150}{5.8/100} = 19{,}850 \text{ cpm per microgram of PBI}$$

$$\frac{10{,}958{,}000 \text{ cpm injected}}{19{,}850 \text{ cpm per microgram of PBI}} = 552 \text{ μg. (EOI pool)}$$

The product of the EOI pool and the turnover rate (k) gave the degradation rate in micrograms of organic iodine per day.

552 μg. (EOI pool) × 10.4% per day (k) = 58 μg. I per day.

This figure represented the rates of formation and degradation, which are identical in a steady state with constant EOI pool. The calculations depend upon the assumption that a steady state existed, which appears reasonable in the absence of significant alterations in the clinical condition.

Clinical material

The subjects selected for study consisted of the following groups:

Normal. Eight healthy medical student volunteers.

Thyrotoxicosis. Six clinically typical cases, verified by abnormally high plasma PBI concentrations. C. S. was untreated. The other subjects except M. O. were receiving 15 drops of Lugol's solution daily, begun one or more weeks prior to study. M. O. was maintained nearly

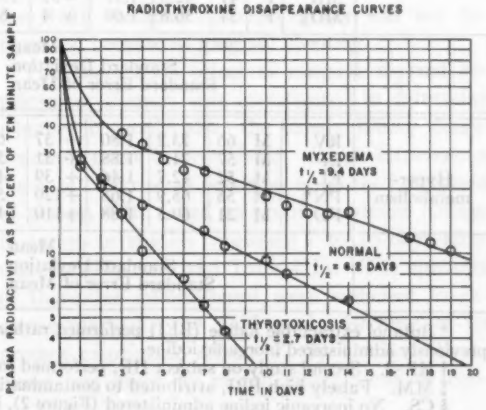

FIGURE 3

Semilogarithmic plot. Studies selected to illustrate kinetic differences.

[8] The relation $k = \frac{\ln 2}{t_{\frac{1}{2}}}$ may be derived from the equation for exponential decay:

$$A = A_0 e^{-kt}$$

where:

A = Radiothyroxine at any time
A_0 = Radiothyroxine at zero time
t = Time in days
$t_{\frac{1}{2}}$ = Half-time
k = Turnover rate as fraction of the body's extrathyroidal organic iodine pool turned over per day. This is conveniently expressed as per cent per day.

TABLE I

Data from radiothyroxine disappearance curves including values adjusted to 1.73 m² surface area and 70 kg. body weight

Condition	Subject	Sex	Age	Wt. (kg.)	Surface area (m^2)	BMR	PBI* (μg. %)	Kinetic and isotope dilution data								
								Kinetics of disappearance		Extrathyroidal organic iodine pool (EOI)			Degradation rate		
								Half-time $t_{\frac{1}{2}}$ (days)	Turnover rate k (%/day)	μg.	μg./1.73 m^2	μg./70 kg.	μg./day	μg./1.73 m^2/day	μg./70 kg./day
Normal	JG	M	23	68.2	1.81		5.2	6.2	11.2	458	440	472	51	49	53
	EG	M	23	75.0	1.94		5.0	5.7	12.2	423	378	395	52	46	48
	AF	M	23	79.5	2.00		7.2	7.7	9.0	625	544	550	56	49	49
	WC	M	23	72.8	1.88		7.2	7.0	9.9	455	419	437	45	41	43
	JL	M	26	81.8	2.06		5.7	7.0	9.9	587	493	505	58	49	50
	RL	M	24	79.1	2.03		5.4	6.1	11.4	507	431	451	58	49	52
	GR	M	24	70.5	1.81		7.0	7.4	9.4	590	566	584	55	53	54
	PR	M	24	84.1	2.04		6.9	6.2	11.2	741	629	615	83	71	69
						Mean		6.7	10.5	548	488	501	57	51	52
						Standard Deviation		0.7	1.1	107	86	77	11	9	8
						Standard Error of Mean		0.3	0.4	38	31	27	4.0	3.1	2.7
Hospital control	RR	M	59	65.5	1.87	− 9	5.8	6.7	10.4	552	511	590	58	54	62
Myxedema	MA	F	58	43.3	1.44	−28	2.0	9.7	7.1	161	193	261	12	14	19
	CC	F	68	60.9	1.60	−44	2.3*	9.3	7.5	276	298	317	21	23	24
	HL	F	40	103.6	2.12	−24	1.9	7.9	8.8	233	191	158	20	16	14
	HR-1	F	61	63.2	1.66	−42	1.0	9.3	7.5	141	146	157	11	11	12
	HR-2†	F	62	46.4	1.47		2.4*	9.4	7.4	233	274	352	17	20	26
	CL	M	74	69.5	1.78	−47	2.2	12.6	5.5	292	283	295	16	16	16
	MM	F	58	89.0	2.03	−49	‡	10.3	6.7						
	CH	F	86	77.3	1.94		2.2	8.3	8.4	264	235	240	22	20	20
	FK	F	69	61.4	1.59		2.0	10.6	6.5	306	334	349	20	22	23
						Mean		9.7	7.3	238	244	266	17	18	19
						Standard Deviation		1.4	1.1	60	64	77	4	4	5
						Standard Error of Mean		0.5	0.4	21	22	27	1.3	1.3	1.8
Thyrotoxicosis	RD	F	31	51.8	1.54	+17	13.6	4.0	17.3	974	1,094	1,315	169	190	228
	LD	F	17	52.3	1.52		14.6	5.2	13.7	954	1,088	1,278	127	145	170
	LS	F	29	54.6	1.52	+68	11.4*	3.7	18.7	1,222	1,393	1,564	229	261	293
	CS§	M	58	60.2	1.76	+74	8.5	2.7	25.6	1,248	1,223	1,448	320	314	371
	MB	F	47	42.3	1.37	+32	13.6	4.7	14.7	880	1,109	1,461	129	163	214
	MO‖	F	54	56.8	1.60	+ 4	8.8	5.8	11.9	845	913	1,039	101	109	124
						Mean		4.4	16.9	1,021	1,137	1,351	179	197	233
						Standard Deviation		1.1	5.0	173	160	185	82	77	88
						Standard Error of Mean		0.5	2.1	70.9	65	75.8	34	35	36
Hypermetabolism	RV	M	66	73.2	1.80	+ 37	6.1	5.2	13.3	810	778	778	108	104	104
	LP	M	57	74.5	1.88	+ 31	5.9	6.2	11.2	1,124	1,034	1,056	126	116	118
	EL	M	52	42.7	1.46	+ 39	4.5	5.2	13.3	605	717	992	81	96	132
	PN¶	M	55	65.9	1.68	+120	5.6	5.4	12.8	540	556	572	69	71	73
	FD	M	21	69.5	1.88	+110	4.8	3.9	17.8	686	631	693	122	112	123
						Mean		5.2	13.7	753	743	818	101	100	110
						Standard Deviation		0.8	2.5	230	183	203	25	18	23
						Standard Error of Mean		0.4	1.1	103	82	91	11	8	10

* Butanol extractable iodine (BEI) performed rather than PBI in CC, HR-2, and LS to avoid interference from previously administered inorganic iodine.

† HR-2. Second study on subject HR, performed four months after cessation of desiccated thyroid therapy.

‡ MM. Falsely high PBI, attributed to contamination, prevented calculations from kinetic data.

§ CS. No inorganic iodine administered (Figure 2), in contrast to other thyrotoxic subjects who received 15 drops of Lugol's solution daily.

‖ MO. Lugol's solution was not administered. Patient was maintained nearly euthyroid with antithyroid medications begun 3 weeks before and continued during the study (Tapazole® 40 mg. daily, later propylthiouracil 400 mg. daily).

¶ PN. Initial high fever (104° F.) subsided on administration of regimen of hydrocortisone 120 mg. daily and triethylene melamine (T.E.M.) 5 mg. daily.

euthyroid for three weeks prior to and throughout the study with Tapazole®, later with propylthiouracil.

Myxedema. Eight clinically typical cases, verified by abnormally low plasma PBI concentrations and by BMR in most instances. Cases H. R. and C. L. were previously untreated. The other patients had received no thyroid replacement therapy for three or more months.

Hypermetabolism without endocrine disease. Four cases of leukemia with fever and one case of urinary tract infection with fever (R. V.). All patients had temperature elevations reaching 104° F. and all had elevated rates of metabolism but normal plasma PBI concentration.

Hospital control. One case of multiple sclerosis with no evidence of endocrine disease and with normal plasma PBI concentration.

In previous studies (1, 6) employing the I^{131} label for turnover rates, Lugol's solution or potassium iodide had been administered to minimize thyroid uptake and promote urinary excretion of I^{131} liberated on degradation of the labeled compound. This precaution of iodine prefeeding to prevent reutilization of the label and its reappearance in the circulation as newly formed radiothyroxine was not carried out by Ingbar and Freinkel (2) whose data show no evidence of significant interference from reutilization, except in hyperthyroid subjects. Sufficient reutilization to affect radiothyroxine disappearance curves significantly would not be expected in myxedema because of negligible thyroid activity, or even in normals, because of mathematical considerations of the turnover and magnitude of the thyroidal iodine pool (3). This inference receives support from the data of R. R., the hospital control subject with multiple sclerosis; the addition of potassium iodide midway through the study produced no visible change in the disappearance curve (Figure 1). Other similar findings, not included in the present report, bear out this conclusion. Moreover, the other euthyroid control subjects in the present work received no added iodine, and had disappearance curves which did not differ significantly from those in the euthyroid series previously reported (1), where iodine was administered.

On the other hand, the curve obtained in the hyperthyroid subject C. S. studied without iodine prefeeding shows a break after the eighth day (Figure 2). The subsequent flattening of the curve was attributed to secretion of newly synthesized labeled hormone which evidently may be quite appreciable in untreated thyrotoxicosis. Consequently, the other thyrotoxic subjects, except M. O., were given 15 drops of Lugol's solution daily prior to and during the studies, and must be considered in partial though slight remission. Subject M. O. was maintained nearly euthyroid for three weeks prior to and throughout the study with Tapazole®, later propylthiouracil. The euthyroid and myxedematous subjects were not given added iodine.

RESULTS

The disappearance curves (Figure 3) showed a slower than normal rate of fall in myxedema, and an accelerated turnover rate in thyrotoxicosis. The extrathyroidal organic iodine (EOI) pools were diminished in myxedema and increased in thyrotoxicosis (Tables I and II).

TABLE II

Summary of mean values for groups studied (adjusted to surface area of 1.73 m^2)—figures represent means ± standard deviations

Group	EOI pool (μg.)	Turnover rate (%/day)	Degradation rate (μg. I/day)
Normal	488 ± 86	10.5 ± 1.1	51 ± 9
Myxedema	244 ± 64	7.3 ± 1.0	18 ± 4
Thyrotoxicosis	1,137 ± 160	16.9 ± 5.0	197 ± 35
Hypermetabolism	743 ± 183	13.7 ± 2.5	100 ± 18

The absolute degradation rates, being the products of the pools and turnovers, showed even wider divergences between the different groups.

Within the hyperthyroid group, the highest values occurred in C. S., the untreated patient, while the lowest were seen in M. O., the patient who was maintained nearly euthyroid with antithyroid medication.

The cases of hypermetabolism without endocrine disease exhibited degradation rates lower than the thyrotoxic patients but still significantly greater than normal. The highest values in a normal subject, P. R., approximated the lowest rate in a hypermetabolic leukemic patient, P. N. In the latter case, hydrocortisone and triethylene melamine (T.E.M.) therapy were instituted shortly after the start of the study, and the temperature declined from 104° F. to 100° F.; whether or not the BMR declined correspondingly was not determined because of gingival bleeding.

The short term effect of massive thyroid replacement therapy was investigated to determine whether it would alter the slowly falling disappearance curve in myxedema. Large intravenous doses of l-thyroxine resulting in marked elevation of plasma PBI failed to produce evident change in curves in studies continued 7 to 10 days after therapy (Figure 4 and Table III). The amounts of thyroxine administered were sufficient to increase markedly the EOI pool. Because of its prompt calorigenic effect (7, 8) L-triiodothyronine [4] was given by mouth either alone or con-

[4] L-triiodothyronine was supplied through the courtesy of Smith, Kline and French Laboratories, Philadelphia, Pa.

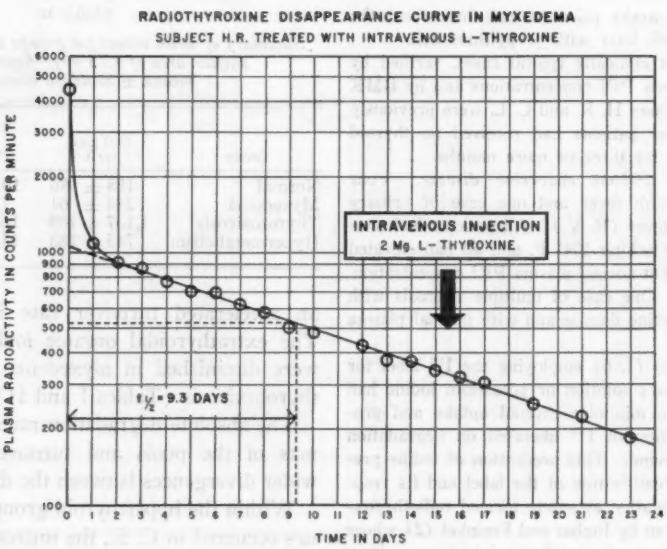
RADIOTHYROXINE DISAPPEARANCE CURVE IN MYXEDEMA
SUBJECT H.R. TREATED WITH INTRAVENOUS L-THYROXINE
PLASMA RADIOACTIVTY IN COUNTS PER MINUTE
TIME IN DAYS
INTRAVENOUS INJECTION
2 Mg. L-THYROXINE
t½ = 9.3 DAYS

FIGURE 4

currently with intravenous thyroxine, again without effect upon radioactivity disappearance curves, although the patients exhibited definite clinical responses.

DISCUSSION

The degradation rates referred to in the present study are so designated, implying rates of removal of hormone from the extrathyroidal pool without

TABLE III

Treatment of myxedema patients *

Patient	EOI pool (*micrograms*)	Therapy	Maximal rise in PBI above baseline (*μg./100 ml.*)	Maximal rise in BMR above baseline
M. A.	161	1 mg. T4; 1.45 mg. T3 over course of 8 days	12.4	
C. C.	276	0.825 mg. T3 over course of 7 days	0.0	
H. L.	233	2 mg. T4; 1.65 mg. T3 over course of 10 days	19.5	
H. R.–1	141	2 mg. T4	Contaminated	+40
H. R.–2	233	2 mg. T4; 1.45 mg. T3 over course of 9 days	>8.8†	
C. L.	292	2 mg. T4; 1 mg. T3; 0.5 mg. T3 (3 days later)	26.2	+33
M. M.		2 mg. T4	23.7	
F. K.	306	2 mg. T3 over course of 7 days	Not determined	

* T4 represents single intravenous injection of L-thyroxine. Thyroxine is 65.4 per cent iodine by weight. T3 represents L-triiodothyronine by mouth, either in a single dose or over the course of several days as noted. Triiodothyronine is 58.5 per cent iodine by weight.

† The increase of 8.8 was obtained on plasma drawn the following day, rather than 10 minutes after intravenous injection of thyroxine, as in the other cases.

specification of the precise pathways of removal. The calculations, in all except three subjects, were based upon plasma PBI determinations, which give slightly higher values than BEI, evidently a more accurate measurement of circulating thyroid hormone (5). The general relationship between level of thyroid function and rate of peripheral degradation of hormone is apparent from the present study as well as the work of Berson and Yalow (3) and of Ingbar and Freinkel (2).

Figure 5 illustrates that degradation rate was related to the plasma PBI concentration, the points showing a considerable scatter in the higher ranges. This scatter was not greatly improved by employing a log-log plot of degradation rate as a function of the square of PBI concentration (3).

It does not necessarily follow, however, that the rate of degradation is controlled directly by the PBI concentration as a mass action effect. On the contrary, the failure of large doses of thyroxine, triiodothyronine, or both to alter the disappearance curve in myxedema suggests that, under given circumstances, a fixed proportion of the extrathyroidal organic iodine pool is removed per day, even when the pool is acutely increased by intravenous thyroxine. This fixed rate persisted, even with elevation of the BMR to normal in *short term* triiodothyronine experiments. Whether the kinetics would be altered by several weeks' or months' maintenance in the euthyroid state remains to be determined. It is conceivable that the mechanism for degrading thyroxine might, like the changes in the integument, require many months of replacement therapy before appreciable reversion to normal.[5]

The unaltered disappearance curves (and hence unaltered daily fractional removal rates) observed after large intravenous doses of thyroxine indicated a markedly elevated *absolute* amount of hormone degraded, since the administered thyroxine greatly exceeded the amount originally present (*cf.* EOI pools, Table III). This finding of unchanged kinetics despite markedly increased pool size and serum concentration of hormone is analogous, perhaps, to the observation by Hellman, Bradlow, Adesman, Fukushima, Kulp, and Gallagher that hydrocortisone-4-C^{14} administered to an oöphorectomized adrenalectomized patient disappeared at the same rate, whether given as a tracer or with 100 mg. hydrocortisone (9).

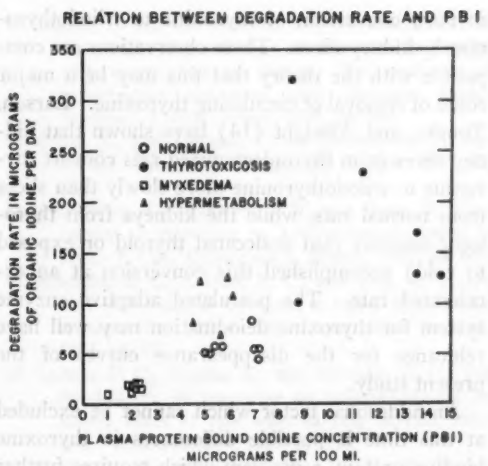

FIGURE 5

Ingbar and Freinkel (10) have reported the converse finding, namely, persistently elevated degradation rates in thyrotoxic patients several months after surgical or antithyroid therapy with attainment of the euthyroid state. In the present study patient M. O. had experienced almost complete remission of her thyrotoxicosis under treatment with antithyroid drugs, but showed a significantly elevated degradation rate, although it was the lowest of the hyperthyroid group.

In considering radiothyroxine turnover studies, attention should be given to the possibility that thyroxine may exert its metabolic effect only after conversion to triiodothyronine in the peripheral tissues. Gross and Leblond (11) demonstrated that "unknown I" (later shown to be triiodothyronine) was formed from thyroxine in peripheral tissues of thyroidectomized animals. Pitt-Rivers, Stanbury, and Rapp (12) found labeled triiodothyronine in the plasma of athyreotic patients after administration of I^{131}-labeled thyroxine. Albright, Larson, and Tust (13) showed

[5] Addendum since completion of paper: Ingbar and Freinkel have observed (personal communication) that the slow thyroxine turnover of myxedematous subjects has increased to normal after several months' maintenance in the euthyroid state with desiccated thyroid therapy. Moreover, they have demonstrated increased thyroxine turnover in normal subjects maintained hypermetabolic (BMR + 25 to + 30) for two to three months with triiodothyronine.

in vitro conversion of thyroxine to triiodothyronine by kidney slices. These observations are compatible with the theory that this may be a major route of removal of circulating thyroxine. Larson, Tomita, and Albright (14) have shown that kidney slices from thyroidectomized rats convert thyroxine to triiodothyronine more slowly than slices from normal rats, while the kidneys from thyrotoxic animals (fed desiccated thyroid or exposed to cold) accomplished this conversion at an accelerated rate. The postulated adaptive enzyme system for thyroxine deiodination may well have relevance for the disappearance curves of the present study.

An additional factor which cannot be excluded at this time is possible differences in thyroxine binding protein, a problem which requires further study.

Elevated degradation rates occurred in the hypermetabolic subjects with normal plasma PBI concentration. This finding of dissociation between PBI and degradation rate would argue further against the assumption that the rate of removal of hormone is a mass action phenomenon determined directly by PBI concentration. Moreover, Castor and Beierwaltes (15) have reported depression of PBI concentration in human subjects made hypermetabolic by administration of dinitrophenol for two days, an observation which could be interpreted as acutely accelerated thyroxine degradation. In the absence of more extensive information with regard to mechanisms of hormone removal, the data would suggest that peripheral tissue metabolism, or some function thereof, has major importance in determining hormone degradation rate.

SUMMARY

1. The rate of peripheral degradation of circulating thyroid hormone was studied with I^{131}-labeled L-thyroxine in normal subjects, patients with thyroid disease, and hypermetabolic patients without endocrine disease (leukemia, fever).

2. After intravenous injection of a tracer amount of radiothyroxine the rate of disappearance of plasma radioactivity was determined.

3. The slow exponential component of the disappearance curve was interpreted as the rate of metabolic degradation.

4. The extrathyroidal organic iodine (EOI) pools calculated by the isotope dilution principle were diminished in myxedema and increased in thyrotoxicosis, as compared with the normals.

5. The turnover rates of extrathyroidal hormone obtained from the half-times of disappearance were slower than normal in myxedema, and accelerated in thyrotoxicosis.

6. The absolute organic iodine degradation rates, being the products of the pools and turnovers, showed even wider divergences between the different groups. The mean values ± standard deviation in micrograms of iodine per day (adjusted to 1.73 m^2 surface area) were as follows:

Normal	51 ± 9
Myxedema	18 ± 4
Thyrotoxicosis	197 ± 35
Hypermetabolism	100 ± 18

7. The hypermetabolic subjects without endocrine disease had increases in pools, turnovers, and degradation rates, although less pronounced than in thyrotoxicosis. The elevated values in nonspecific hypermetabolism suggested that peripheral tissue metabolism, or some function thereof, has a major role in determining hormone degradation rate.

8. Administration of large amounts of thyroxine or triiodothyronine or both to myxedematous subjects failed to alter the shape of the disappearance curve. This indicated turnover of a fixed proportion of the EOI pool daily, during short term studies, despite marked elevation of the plasma PBI concentration, enlargement of the EOI pool, and increase in the absolute organic iodine degradation rate.

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CHARACTERIZATION OF THE PROTEINS OF HUMAN SYNOVIAL FLUID IN CERTAIN DISEASE STATES [1]

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(Submitted for publication February 20, 1956, accepted April 3, 1956)

In search of the similarities and differences between blood plasma and synovial fluid, on one hand, and normal and pathological joint fluid on the other, it appeared important to investigate systematically the protein components of joint fluid. Investigations of the synovial fluid proteins by electrophoresis have been carried out by several authors (1-4). The identification of these proteins has been initiated (1, 5). Fractionation with ammonium sulfate to obtain the albumin-globulin ratio (6) and demonstration of definite properties of certain proteins by immunochemical methods and enzymatic reactions have been reported (1, 5).

This publication presents a study on the characterization of the proteins in pathological synovial fluids.

MATERIALS AND METHODS

The collection of synovial fluid and plasma as well as the procedure for the fractionation of their proteins have been described in a previous publication (7). All fluids were drawn from knee joints of patients with rheumatoid or traumatic arthritis or neuroarthropathy, and were obtained through the courtesy of the clinical staff of the Arthritis Unit. Most of the fluids deposited a precipitate within 20 hours although acid-citrate-dextrose (ACD) solution had been added. Prior to fractionation the joint fluids were treated with hyaluronidase (7).

The *electrophoretic and ultracentrifugal analyses* were carried out in a Perkin-Elmer electrophoresis apparatus and a Spinco Model E ultracentrifuge, respectively. *Paper electrophoresis* was performed essentially according to the method of Grassmann and Hannig (8, 9). After being heated at 110° for 10 minutes, the paper strips were stained with Amidoschwarz 10B.[2] The intensity of the color was read in an "Elphor" densitometer.[2] The relative percentage of the separated proteins was determined planimetrically from the curve obtained by plotting the optical density as a function of distance on the paper strip. A correction factor of 1.5 was applied for orosomucoid (10, 11), of which the polypeptide moiety was 66 per cent.

Immunochemical methods: For the *immunoelectrophoresis* described by Grabar and Williams (12) a plate glass (25 × 25 cm.) was used as support. Plasticin (1.2 cm. high) was mounted approximately 2.5 cm. from the edges of the plate. The plate glass was carefully warmed and 20 ml. of pH 8.6, Γ/2 0.07 borate buffer containing 2 per cent agar were added so as to obtain an even layer, (to 1 volume of 4 per cent aqueous agar solution, Bacto Agar, Difco, extracted with distilled water for 24 hours prior to heating, 1 volume of borate buffer was added; the borate buffer consisted of 1 part of 0.07 M $Na_2B_4O_7$ and 2 parts 0.07 M H_3BO_3; borate buffer was preferred because of its inhibitory effect on the growth of molds). After cooling to room temperature, 6 plastic pieces (0.6 × 1.2 × 15 cm.) were arranged on the agar parallel to each other and to one edge of the plate. Their ends were 5 cm. away from the next edge, thus forming 5 spaces of 3 cm. width and 15 cm. length. Next, 200 ml. of the same agar solution were added giving a thickness of 0.8 to 1.0 cm. After the plate had again been cooled to room temperature, the plastic pieces were loosened with a spatula and carefully removed. The resulting 6 troughs were used after electrophoresis to receive the antiserum. In the center of each section (3 × 15 cm.) which was separated by the troughs, a small reservoir (1.2 cm. long, 0.25 cm. wide and 0.9 cm. deep) was cut out. The main axis of the reservoirs was perpendicular to the troughs and one or two drops of liquid borate-agar was added to each to seal any breaks. Then, the protein solutions (serum, synovial fluid digested with hyaluronidase, 4 per cent human serum albumin, and 1.2 per cent human γ-globulin), previously dialyzed against the same borate buffer, were mixed at 52°C with an equal volume of 4 per cent aqueous agar solution. The individual reservoirs were filled exactly to the level with one of the protein solutions. The agar plate was then placed in the electrophoresis box (outer dimensions: 38 × 26 × 12 cm.; buffer chambers 26 × 6.7 × 3.4 cm.; center compartment 26 × 26 × 5.5) and transferred to a 2°C room. Four layers of

[1] This is publication No. 192 of the Robert W. Lovett Memorial Laboratories for the Study of Crippling Diseases, Harvard Medical School, Boston, Massachusetts. Grants in support of these investigations have been received from the Helen Hay Whitney Foundation, New York City, and from the National Institutes of Health, U. S. Public Health Service, A-509. A preliminary report on this work has been published by Karl Schmid and Edith C. Rosa, Federation Proc., 1955, **14**, 276.

[2] Bender and Hobein, Munich, Germany.

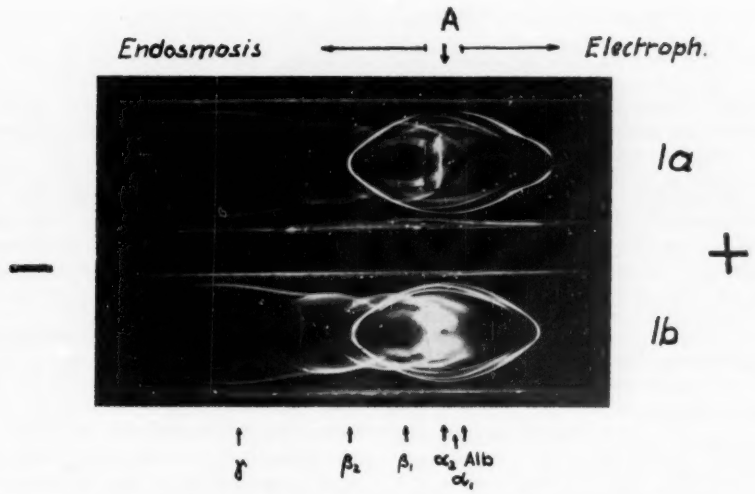


FIG. 1. IMMUNOELECTROPHORESIS OF TRAUMATIC SYNOVIAL FLUID (a) AND SERUM (b) FROM THE SAME INDIVIDUAL

Joint fluid had been treated with hyaluronidase prior to the experiment. A indicates the position of application. Electrophoresis in borate buffer, pH 8.6, Γ/2 0.07, containing 2 per cent agar, for 17 hours at 180 V and 40 mA. The arrow "Electroph." indicates the direction of the electrophoretic movement of the proteins, and the arrow "Endosmosis" indicates the direction of the electroendosmosis. After the electrophoresis was completed, the troughs between the agar sections were filled with horse serum against human serum. Therefore, the precipitin zones formed symmetrical patterns. The positions of albumin and γ-globulin were found by applying these proteins as references on adjoining sections. The precipitin zones of synovial fluid (a) appeared less intense than those of the serum because of its lower protein concentration. The line corresponding to albumin, however, was very distinct due to the relatively high albumin concentration in this joint fluid.

Whatman No. 1 paper (24 cm. wide) soaked in the borate buffer were used as electric connection between the ends of the agar plate and the adjoining borate buffer reservoirs. The buffer chambers, in turn, were again connected to holes filled with glass wool with a second set of buffer chambers in which the electrodes were placed. The electrodes were made by winding 120 cm. of stainless steel wire (0.05 cm.) around a glass tube with diameter of 1.5 cm. To secure an even level of the buffer in all 4 compartments, the two inner reservoirs were connected with each other by a thin rubber tubing. After half an hour of temperature equilibration, 180 volts were applied giving a current of 40 mA. The electrophoresis was continued for 17 hours. Owing to strong electroendosmosis, acting in the direction opposite to the electrophoretic movement, albumin remained almost exactly at the position of application, and γ-globulin appeared to have moved against the direction indicated by the electrophoretic mobility (Figure 1). Between the albumin and γ-globulin, the α_1-, α_2-, β_1-, and β_2-globulins were distributed according to their electrophoretic mobilities. During electrophoresis, diffusion occurred so that each group of globulins would appear as a band, broader than the reservoir where the protein solution was originally applied. Under these circumstances, the albumin, α_1- and α_2-globulin did not appear to be well separated. After electrophoresis, the plate was brought to room temperature, the troughs filled with the antiserum, and the system kept in an air-tight box to prevent dehydration. The proteins of the antiserum diffused into the two adjoining agar sections at approximately the same rate, at each point along a line parallel to the troughs. The proteins of the analysis, now separated according to their electrophoretic mobilities, diffused further toward the troughs, as well as in the direction of the main axis of the agar sections. At the point where the corresponding antibodies and antigens met (point of equivalence), a complex of these two components was formed and precipitated as lines. Such complexes are known to be soluble in excess of either antibody or antigen. Thus, depending on their concentration, different amounts were transported by diffusion toward the precipitin zones, which were dissolved, and new ones formed (assuming that the precipitin zones did not present an obstacle to diffusion). Consequently, one observed that these zones moved either toward or away from the troughs. The precipitin zones

appeared as elliptical curves because the concentration of the antigens varied from zero over a maximum to zero when observed along a line parallel to the troughs. For the exact interpretation of the precipitate lines see references 12–14. After 6 hours, the first faint line corresponding to albumin became visible, and within 6 days all lines had formed. The antiserum which diffused into the agar sections cleared the turbidity of the agar plate and facilitated the taking of pictures. The number of precipitin zones indicated the minimal number of antigen components. Using this particular antiserum, 12 lines were observed. The pictures were taken in the following way: A beam of light illuminating the agar section with the precipitin lines, was projected from an angle beneath the plate. In this way the precipitate lines showed up against the dark background. Care was taken to avoid dust. The agar plate was washed with soft paper previously soaked in saline.

For the *Ouchterlony* test (15), 10 ml. of the above mentioned agar-borate solution was poured into each petri dish and allowed to gel. After prolonged cooling, rubber-serum stoppers (1.4 cm. outer diameter) perforated with 21 gauge hypodermic needles were placed on each plate, one in the center and usually four more at distances of 1.6 cm. from the center and at equal distance from each other. Next, 25 to 30 ml. more agar solution was added. When thoroughly cooled, the stoppers were removed and 2 to 3 drops of agar were added to each reservoir to seal off any breaks. In our experiments, the antiserum was placed in the center reservoir and the test solutions in the others. The formation of the zones is governed by the same principle as described in the method of Grabar. The exact interpretation, particularly of the complications, has been further studied by Burtin (13) and Wilson and Pringle (14). It was concluded from the precipitin zones which formed closed systems (fusion of the precipitate lines), that the corresponding proteins were identical.

Furthermore, the technique described by *Oudin* (16) was applied to certain protein fractions to identify the γ-globulins. For the titration of the *antistreptolysin-O* content and for the determination of the *diphtheria antitoxin* content (rabbit skin test), the standard methods (17, 18) were used.

Ceruloplasmin (19) was determined as follows: Fraction III was dialyzed at 2°C against distilled water for 14 hours to remove the ethanol. Insoluble material was centrifuged off. The optical density of the supernatant solution was read against water between 580 and 630 mμ at intervals of 10 mμ, in a Beckman DU spectrophotometer using a 1-cm. cell. An aliquot of 3.0 ml. of the supernatant solution was mixed with 0.2 ml. of a 1 per cent KCN solution, and its optical density read in the same way after standing for 5 hours at room temperature. For the calculation of the percentage of ceruloplasmin, the difference in optical densities at 610 mμ, representing the maximal absorption of this protein in the visible spectrum, was divided by the extinction coefficient of 0.68 ($= E^{1\%}_{1\,cm}$). Appropriate corrections for dilution occurring during fractionation, dialysis, and addition of the KCN solution were applied. The corresponding fraction derived from normal human plasma was measured simultaneously. This procedure can obviously be simplified, when sufficient material is available, by reading the dialyzed and centrifuged Fraction III against an aliquot of the same solution which had previously been treated with KCN.

Lipoproteins expressed as cholesterol were measured by the method of Bloor, Pelkan, and Allen (20).

The *β_1-metal-combining protein* determinations (21, 22) were carried out as follows: 500 ml. of citrate barbiturate buffer, pH 8.6, Γ/2 0.1, commonly employed in electrophoresis, were added to 37.5 ml. of acetate buffer, pH 4.0, Γ/2 0.1, to obtain a pH of 7.4. After standing over night in the cold room, the precipitated barbituric acid was separated, and the supernatant solution was mixed with 20 ml. of freshly prepared 20 per cent $Fe(NH_4)_2(SO_4)_2$-solution. Aliquots of Fractions IV and V were dialyzed for two days against this buffer. The intensity of the developed pink color ($E^{1\%}_{1\,cm} = 0.57$) was measured at 465 mμ, part of the same protein solutions acidified with 0.1 ml. of acetic acid per ml. being used as a blank.

Albumin was measured by the method of Rutstein, Ingenito, and Reynolds (23) which involved the determination of the capacity of albumin to bind 2-(4′-hydroxybenzeneazo) benzoic acid (HBABA).

RESULTS

For the characterization of the synovial fluid proteins, two fundamentally different approaches were chosen. First, the proteins were identified by precipitin reactions after separation by electrophoresis in agar (method of Grabar). Second, the proteins were separated into six fractions by means of solubility differences (7), and were then characterized by chemical and physico-chemical methods and by the measurement of definite properties of proteins known to occur in blood plasma. In contrast to the first method that permits qualitative measurements only, the second makes possible qualitative and quantitative determinations.

1. *Characterization of the synovial fluid proteins by immunoelectrophoresis*

Synovial fluids from patients with rheumatoid arthritis or knee injuries together with the plasma from the same individuals were investigated simultaneously. The results obtained were consistent: The characteristics, number and distribution of precipitin zones were the same for each set of fluids, and suggested that the proteins of plasma also occur in synovial fluid as judged by these characteristic tests. The precipitin zones were

grouped according to the electrophoretic mobilities of albumin, α_1-, α_2-, β_1-, β_2, and γ-globulin, but only the zones corresponding to albumin and γ-globulin were identified. It is important, however, to note that the immunoelectrophoretic patterns obtained from joint fluid and plasma of the same patient were identical. A typical experiment is illustrated in Figure 1.

2. *Characterization of the synovial fluid proteins after separation by the low temperature—low salt—ethanol procedure*

Joint fluids and plasma from the same individuals were fractionated simultaneously.. A proper comparison could thus be obtained as well as a test of the reliability of the method, for which the results from normal human plasma were used as references (*7*). Two characteristic experiments are reported as examples.

a. *Synovial fluid from a patient with rheumatoid arthritis:* Synovial fluid obtained from a patient (W. W. 745536) with rheumatoid arthritis was centrifuged at approximately 12,000 r.p.m. for one hour, yielding a straw-yellow, viscous fluid. Since fractionation was not carried out immediately, the fluid was kept at − 30° for two months. After being thawed the clear fluid was digested with hyaluronidase. A clot which formed within 14 hours was centrifuged off. ACD-plasma from the same individual was also kept at − 30° for the same time. The electrophoretic distribution of the proteins of the original fluid and plasma was identical.

For the fractionation, 28 ml. of ACD-synovial fluid and 20 ml. of ACD-plasma were used so that each contained approximately equal amounts of protein. Essentially the same results (Table I) were obtained from both joint fluid and plasma: The protein content of each of the six fractions (except Fraction I of synovial fluid because of its previous clotting), the content of protein-bound hexose, the distribution of the cholesterol, the electrophoretic distribution and the mobilities of the proteins and the distribution in the ultracentrifuge, as well as the calculated sedimentation constants, were within experimental errors found to be alike for both sets of fractions.[3] The percentages of γ-globulin in Fraction I and III were very similar to those of the corresponding fractions from normal human plasma (*7*).

In addition to these investigations, during which the proteins were studied from the point of view of general characteristics, except for the cholesterol and in part the carbohydrate content, experiments were carried out for the determination of definite properties of certain protein components known to occur in plasma. Again, these experiments were performed simultaneously with the fractions from both synovial fluid and plasma. The clotting of *Fraction I* indicated the presence of fibrinogen. The clear supernatant solution was kept at + 2°C for 20 hours resulting in the formation of a precipitate. The solubility of the precipitated material at room temperature proved the presence of "cold-insoluble globulin" (*24*). *Fraction II,* in which the γ-globulins were concentrated, was analyzed for specific antibodies. The antistreptolysin-O content, of which normal human plasma contains between 50 and 250 standard units per ml. amounted to 45 U per ml. of ACD-synovial fluid. However, the protein concentration of the latter fluid was only 5.7 per cent. The antistreptolysin-O titre in both Fractions II were almost equal. Diphtheria antitoxin showed the same distribution, namely, the activity of the proteins in each of the two Fractions II was approximately half of the total. The presence of ceruloplasmin precipitated in *Fractions III* was evidenced by its blue color after incubation of an aliquot for 10 minutes at 70°C. Fraction III of synovial fluid and of plasma exhibited the same isoagglutinin titre. The content of the β_1- and α_1-lipoproteins, judged by the cholesterol content of Fraction III and *Fraction IV,* was almost equal for both fluids. Fraction IV contained approximately 20 per cent of the α_2-globulins; the rest had previously been precipitated in Fraction III. The estimation of the α_2-globulins in Fraction III from the electrophoretic pattern might be erroneous owing to the change in the mobility of the β_1-lipoproteins due to autoxidation (*25*). The amount of β_1-metal-binding protein concentrated in *Fraction V* was the same for both fluids, amounting to 3 per cent, while Fraction IV was free of this component. The albumin of Fraction V, which was marked by a distinct yellow color and its low content of bound hexose, exhibited the same capacity to bind

[3] These sedimentation constants were identical to those reported for the corresponding fractions from normal human plasma.

TABLE I

Fractionation of synovial fluid from a patient with rheumatoid arthritis (W. W. 745536)

	Fraction	Total protein* mg.	Total hexose mg.	Total hexose %	Total cholesterol mg.	Electrophoresis at pH 8.6 (Expressed in relative percentage) Alb.	α_1	α_2	β_1	β_2+ Fibr.	γ	$S_{20,w}$†	Total albumin HBABA mg.	Total β_1-metal-combining protein mg. (%)	Total anti-streptolysin-O	Total diphtheria antitoxin	Titer of isoagglutinins‡	Electrophoresis at pH 4.5 (Relative percentage)
28 ml. ACD-Synovial Fluid	Fluid	1,610(5.8%)			28	55	5	7	15	5	13		860		1,260 U	2.8 U		α_1 1% α_2 3% Rest 96%
	I	64						clotted										
	II	170	1.9	1.1		1			3	20	76	6.8			400 U	0.6–2.0 U	inactive	
	III	340	12	3.7	17	5		25	39	20	11						Anti-A 1:64 Anti-B 1:8	
	IV	94	1.1	1.2	2.8	44	24	15	10	2	5	4.6§		0				
	V	900	6.4	0.9		92	1	1	6			4.1	720	24(3)				
	VI	27										2.9§						
20 ml. ACD-Plasma	Plasma	1,550(7.7%)			24	55	4	8	14	7	12		640					α_1 1% α_2 3% Rest 96%
	I	110						clotted										
	II	140	2.4	1.7		4			18		78	6.9			560 U	0.2–0.6 U	inactive	
	III	300	8.6	2.9	15	4	7	24	37	16	12						Anti-A 1:64 Anti-B 1:8	
	IV	110	4.1	3.6	3	31	37	22	6		4	4.6§		0				
	V	730	5.3	0.7		92	2	1	5			4.0	450‖	21(3)				
	VI	27										2.9§						

* Biuret.

† In 0.15 M NaCl at a protein concentration of approx. 1 per cent.

‡ Both Fractions III adjusted to 20 ml.

§ Sedimentation constant of heavier component not indicated.

‖ The value of 450 mg. albumin determined by the HBABA-method seems to be erroneous, because 670 mg. were expected from measurements of the protein content of Fraction V by the biuret method and electrophoresis. We feel that this discrepancy is not significant in view of the other measurements (solubility, electrophoretic distribution and mobility, ultracentrifugal distribution and sedimentation constants, immunochemical analyses, and carbohydrate content) carried out on the two Fractions V. In addition, the albumin content of other plasma and their Fractions V found by the HBABA-method agreed with that obtained from protein content and electrophoresis.

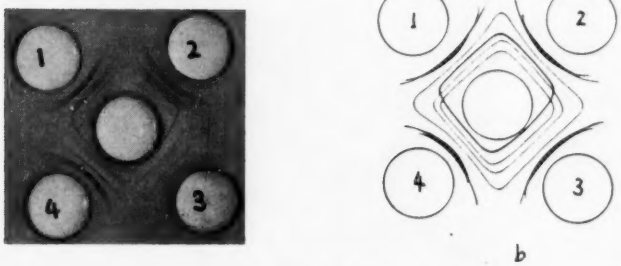


FIG. 2. OUCHTERLONY PLATE (2a) OF FRACTION III OBTAINED FROM ARTHRITIC SYNOVIAL FLUID

Figure 2b represents a sketch of Figure 2a. Reservoir 1 contained Fraction III of this synovial fluid, reservoir 2 Fraction III of the same patient's plasma, reservoir 3 normal human plasma, reservoir 4 Fraction II + III of normal human plasma and center reservoir horse serum against human serum.

HBABA as normal human serum albumin is known to have (23). The presence of α_1- and α_2-glycoproteins in *Fraction VI* was shown by their low sedimentation constant. The acid isoelectric points (10), indicated by electrophoresis of the original fluids at pH 4.5, conform with this view.

Fractions II, III, IV, and V were also investigated for identity of the proteins by the method of Ouchterlony. The corresponding fractions of synovial fluid and plasma from the same patient as well as that of normal human plasma were put on the same plate. The concavities for these four solutions were arranged on the plate at equal distances from the center one in which horse serum active against human serum was placed. It was again concluded that most of the proteins in the corresponding fractions were identical, as judged by the precipitin zones which formed closed systems (Figure 2a). The γ-globulin content of Fractions III and Fractions IV, amounting to a small percentage of the total as judged by electrophoretic analysis, was identified with a specific rabbit serum against human γ-globulin with use of the technique of Oudin.

For the experiment described in section 2a, a patient with rheumatoid arthritis was chosen who showed severe knee involvement, and whose synovial fluid and plasma revealed essentially the same patterns upon electrophoresis. During this study, joint fluids from six other patients with rheumatoid arthritis or neuroarthropathy were also fractionated. They were characterized by their low content of the α_2-globulins and increased content of γ-globulins, compared with the plasma of the same individuals (26). The results obtained from investigating the protein fractions of these joint fluids were essentially the same as those reported in the previous experiment. If the γ-globulin content was 20 per cent or higher, only part of it could be extracted from Fraction II + III into Fraction II.

b. *Synovial fluid from a patient with traumatic arthritis:* Synovial fluid and serum obtained from a healthy individual (RBJE) 2 days after an injury to his knee were centrifuged and stored at $-30°C$ until fractionation was carried out. Since the volume of the serum was too small to perform a complete analysis, particularly fractionation, plasma from a different healthy individual was fractionated simultaneously. Prior to the fractionation, the synovial fluid was digested with hyaluronidase. The fluid clotted within 20 hours. The electrophoretic distribution of the proteins of the original synovial fluid, distinguished by the high content of albumin and γ-globulin and low content of α_2-globulin, is typical of traumatic joint fluid (26). The electrophoretic pattern of the serum was normal.

The characterization of the synovial fluid proteins after fractionation showed the following results (Table II): The relative amount of protein in the six fractions was essentially the same as that reported for normal human plasma. The low protein content of Fraction I was due to the decreased concentration of fibrinogen, and that

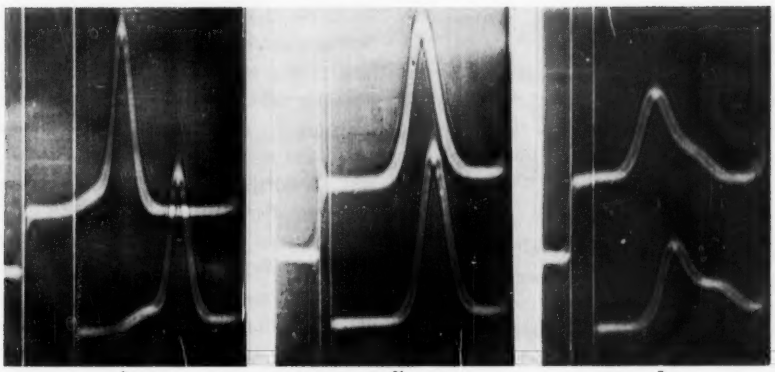
TABLE II

Fractionation of traumatic synovial fluid (R. B. J.)

	Fraction	Total protein* *mg.*	Total cholesterol *mg.*	Electrophoresis at pH 8.6, Γ/2 0.1 (Expressed in relative percentage) Alb.	α_1	α_2	β_1	β_2+ Fibr.	γ	$S_{20,w}$†	Total albumin HBABA *mg.*	Total β_1-metal-combining protein *mg.* (% *protein*)	Total ceruloplasmin *mg.*	Total antistreptolysin-O	Total diphtheria antitoxin	Titer of isoagglutinins‡	Electrophoresis at pH 4.5 (Relative percentage)
	Fluid	910(3.2%)	16	68	3	3	14	3	9		640			930 U	29–90 U	Anti-A 1:8 Anti-B 1:2	α_1 2% α_2 5% Rest 93%
29 ml. Joint Fluid	I	22§															
	II	37		4	3	4	7	17	65	6.4							
	III	220	14		17	4	45	4	30				4.2			Anti-A 1:16 Anti-B 1:1	
	IV	65	1.0	88	6	2	4			4.0¶							
	V	450	<0.3	97	1	1	1			3.8	400	23(2.4)					
	VI	12‖		30	37	18	11	4		2.6¶							α_1 30% α_2 16% Rest 54%
	12 ml. serum	960(8%)	22	55	4	7	19	2	13		580			1,200 U	36–120 U	Anti-A 1:64 Anti-B 1:16	α_1 3% α_2 4% Rest 93%

* Biuret.
† In 0.10 M NaCl at a protein concentration of approx. 1 per cent.
‡ Solution of Fraction III adjusted to 29 ml.
§ Six mg. of fibrin clot.
‖ Determined by weight after lyophilization.
¶ Sedimentation constant of heavier component not indicated.

Electrophoreses of Fraction VI, both at pH 8.6 and 4.5, were carried out on paper; ceruloplasmin content of normal human serum is 0.32 mg. per ml. as found by Scheinberg and Gitlin, Science, 1952, **116**, 484.



3a 3b 3c

FIG. 3. ULTRACENTRIFUGAL ANALYSES OF PROTEIN FRACTIONS FROM TRAUMATIC JOINT FLUID

The analyses were carried out under the following conditions: 0.15 M NaCl, 56100 r.p.m., temperature 19°C to 20°C, protein concentration approximately 1 per cent, direction of sedimentation from left to right. Each picture shows two curves; two samples were run simultaneously by means of a wedge window cell. The upper curve, the meniscus of which is displaced to left due to slightly larger volume of the sample, is that of the sample in the wedge window. The bottom curves represent the synovial fluid fractions and the top curves the corresponding fractions of normal human plasma. Figure 3a, Fraction II (γ-globulin); 73 minutes, wire angle 50°; Figure 3b, Fraction V (albumin), 116 minutes, wire angle 50°; Figure 3c, Fraction VI (glycoproteins), 137 minutes, wire angle 50°.

of Fraction IV perhaps due to the low α_2-globulin concentration in the original fluid. The electrophoretic distribution and the mobilities of the proteins of the six fractions as well as their ultracentrifugal distribution (Figure 3) and sedimentation constants were essentially identical with those reported for normal human plasma (*27*).

Again, additional specific tests were carried out to prove the existence in traumatic joint fluid of protein components present in blood plasma. *Fibrinogen* was detected in the synovial fluid and in Fraction I by the formation of a clot. *Cold insoluble globulin* concentrated in Fraction I was recognized by its extreme insolubility at + 2°C and its relatively high solubility at room temperature. The titres of *diphtheria antitoxin* and *antistreptolysin-O* of both synovial fluid and serum, corrected for protein concentration, were found to be comparable to those reported for average normal human plasma. The content of *ceruloplasmin*, expressed as percentage of the total protein concentration, ranged within the values reported for normal human plasma. The total *cholesterol* content was approximately one-half that of normal human plasma, if calculated on the basis of the total amount of protein. It is important to point out that the cholesterol content of Fractions IV and V, representing the α_1-lipoproteins, was about 12 per cent of the total as compared with 25 per cent of the same fractions of normal human plasma. Similar observations were made by Noble, Boucek, and Kao (*28*) in their study on the lipoproteins of connective tissue. Further investigation by the flotation technique might yield more information about the distribution of the lipoproteins. The content of *β_1-metal-combining protein* was 6 per cent of the protein in Fraction V or 3 per cent of the total protein, which was equal to the value reported for normal human plasma (*29*). The content of *albumin*, measured by its capacity to bind HBABA, was essentially that found by electrophoresis. The presence of *orosomucoid* and other *glycoproteins* of low molecular weights and acid isoelectric points was proven by paper electrophoresis of Fraction VI (Figure 4) and by free electrophoresis of the original joint fluid, both at pH 4.5. Furthermore, the high content of protein-bound hexose of this

fraction, amounting to 10 per cent, equaled the corresponding value of Fraction VI of normal human plasma. The identity of the proteins in traumatic synovial fluid was further confirmed by immunochemical analyses using the Ouchterlony technique described in section 1.

DISCUSSION

For the characterization of a protein, it has become clear that the results obtained from one method only can never establish its identity. Because of the complexity of the protein molecule, a series of methods based on different principles should be included in such a study.

For the characterization of the synovial fluid proteins, the proteins of joint fluid were separated from each other by means of differences in their electrostatic charges as well as in their solubilities. The method of Grabar combined electrophoresis with immunochemical reactions, of which the specificity is extremely high. The procedure for the separation of the proteins into solubility groups, referred to as fractions in Tables I and II, made possible the recovery and further investigation of the proteins. The conditions of precipitation of the proteins represent a precise measure for their solubilities, which are some of their characteristics.

In characterizing the synovial fluid proteins, it was found that the proteins of the corresponding fractions, derived from synovial fluid and plasma of the same individual, exhibited the same solubility properties, the same electrophoretic distribution and mobilities of the respective components, and the same sedimentation constants. Yet, the possibility existed that these proteins were not identical. In order to exclude such a possibility, further independent methods were employed which allowed the measurement of very distinct properties of the proteins such as titres of isoagglutinin, diphtheria antitoxin, and antistreptolysin-O, reaction of ceruloplasmin with cyanide ions, and ion-binding capacity of albumin and β_1-metal-combining protein. It was concluded from the results of the different methods used that, for the most part, the synovial fluid proteins are identical

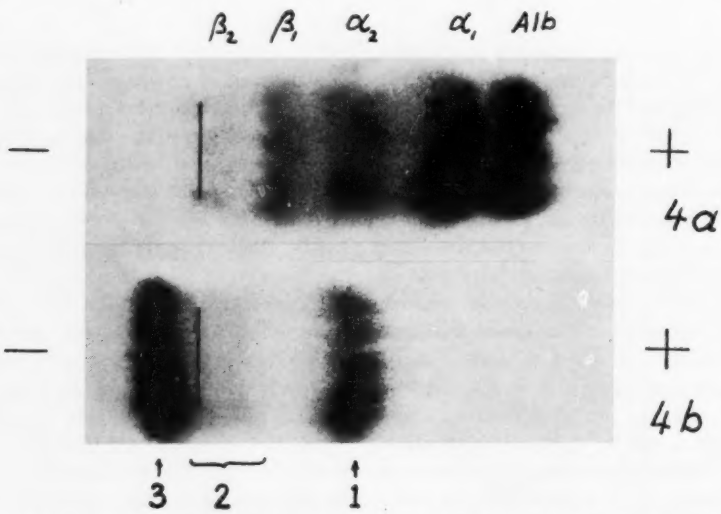


FIG. 4. PAPER ELECTROPHORESIS OF FRACTION VI, DERIVED FROM TRAUMATIC JOINT FLUID, IN DIETHYL-BARBITURATE BUFFER pH 8.6, Γ/2 0.1 (4a) AND IN ACETATE BUFFER pH 4.5, Γ/2 0.1 (4b)

The dark lines indicate the position of application. Staining with Amidoschwarz 10B. Area 1 in Figure 4b is representative of orosomucoid identical with α_1-globulin in Figure 4a; area 2 is identical with most of the α_2-globulin in Figure 4a, and area 3 representing the proteins which were positively charged at pH 4.5, includes albumin, β-globulin, and a minute part of the α_2-globulin.

with those of plasma. It is emphasized that the findings described do not relate to normal synovial fluid which may present a different pattern. The existence of special protein components present solely in synovial fluid is not excluded.

Although the synovial fluid proteins exhibited the same characteristics as the plasma proteins, the following differences were found: First, the differences in total protein concentration and in the relative content, especially of albumin, α_2- and γ-globulins, reported earlier (26), were confirmed. Second, the ratio of β_1- to α_1-lipoprotein, measured as cholesterol, appeared higher than that of normal human plasma.

It should be pointed out that it is a difficult task to establish the identity of two proteins (30). The results obtained from these investigations led obviously to the question of the fine structure of the "identical" proteins, in other words, as to whether or not one individual always synthesizes a certain protein by exactly the same process.

SUMMARY

The proteins of synovial fluid from patients with rheumatoid arthritis, neuroarthropathy, or traumatic injury have been characterized. They are identical with those of plasma as judged by the results obtained from several different methods including electrophoresis, ultracentrifugal analyses, separation by solubility, immunochemical methods, and ion-binding measurements. The following proteins were identified: Fibrinogen, cold insoluble globulin, diphtheria antitoxin, antistreptolysin-O, isoagglutinins, ceruloplasmin, β_1-metal-combining protein, α_1- and β_1-lipoproteins. Albumin, γ-globulin, and glycoproteins of low molecular weights and acid isoelectric points were isolated. Known differences between synovial fluid and plasma were confirmed.

ACKNOWLEDGMENTS

The authors would like to express their appreciation to Dr. Marian W. Ropes and the other members of the clinical staff of the Lovett Memorial Laboratories through whose effort the synovial fluid was made available. Our thanks are also due to Dr. James Heyl of Exeter, N.H., for providing the traumatic joint fluids. We are particularly indebted to Dr. Robert Berg for the gift of horse serum against human serum, to Miss Jessy Hendry, New York State Department of Health, Albany, N. Y., for the gift of horse sera against human γ-globulin and albumin, respectively, and to Dr. Harry Bowen of the Division of Biologic Laboratories, Massachusetts Department of Health, for the antistreptolysin and diphtheria antitoxin determinations. We should also like to thank Drs. D. Gitlin and H. Habich, the Children's Medical Center, for the introduction in the immunochemical methods. The expert assistance of Mrs. Juliet C. McGlynn and Miss Annemarie Ulrich who performed the electrophoretic and ultracentrifugal analyses, is gratefully acknowledged.

Addendum

The designation "orosomucoid" is used for the human glycoprotein previously named "MP-1" by Winzler (11) and "acid glycoprotein" by Schmid (10).

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COMPARISON OF LIVER BLOOD FLOW VALUES ESTIMATED BY THE BROMSULPHALEIN AND BY THE RADIOGOLD METHOD

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(Submitted for publication July 19, 1955; accepted April 3, 1956)

In a previous paper (1) we have described a method of following the disappearance of intravenously injected colloidal radioactive gold from the circulation in man and have discussed the possibilities of calculating liver blood flow from the disappearance rate constant as the fraction of blood volume perfusing the liver per unit time. If liver blood flow values estimated in this way could be demonstrated to be correct this method should have many advantages over other methods currently in use: it does not require hepatic vein catheterization; with only one blood sample withdrawn for determination of hematocrit and plasma volume, it does not involve any undue blood loss to the patient; being exceedingly simple and mechanized to a considerable extent by the use of counting rate meters and recorders, it can be carried out by a single technician; it can be repeated several times without exposing the patient to any risk thereby enabling observation of changes in hepatic circulation over any period of time.

Hepatic blood flow values estimated by this method in 25 normal subjects (18 males and 7 females) were found to average 1310 ml. per min. (1) and thus to be somewhat lower but still within the range of normal values reported by other groups who used the bromsulphalein or other methods. However, even this difference might not have been a real one. In this laboratory the observed venous hematocrit is corrected for trapped plasma and for the uneven distribution of cells and plasma within the whole body circulation. This leads to a blood volume which is about 14 per cent lower than that which is calculated from the uncorrected hematocrit. None of the groups of investigators whose results have been compared with the average normal radiogold results, appear to have used any correction of the hematocrit. It might, therefore, well be that the difference between average hepatic blood flows estimated by these two methods in different groups of normal subjects has been caused by the difference in the methods of calculating blood volume.

However, a few experiments designed to investigate the efficiency of the liver phagocytes to remove the colloid particles from the blood passing through the liver, indicated that the radiogold method underestimates the true hepatic blood flow by almost 20 per cent (1). If this is true a direct comparison of BSP and radiogold results should reveal a real difference in the calculated flow values. Simultaneous estimations of hepatic blood flow by these two methods were, therefore, performed in 15 subjects the results of which are presented in this paper.

METHODS

Material. The subjects studied were hospitalized patients in whom for diagnostic purposes a heart or hepatic vein catheterization was warranted. Therefore, none of the subjects could be considered to be "normal."

Catheterization. In each patient a Cournand catheter connected with a slowly running saline-heparin infusion was first introduced into the pulmonary artery under fluoroscopic control. Two blood samples were taken from there at two different positions and another one from either the brachial or the femoral artery, their oxygen content being subsequently determined with a Van Slyke apparatus. Oxygen consumption was measured with a Knipping spirometer and cardiac output calculated from the data thus obtained. The catheter was then advanced into a right hepatic vein in all patients with one exception where only a left hepatic vein could be reached. Following the estimation of hepatic blood flow by the BSP and by the radiogold method, blood samples from the hepatic vein were taken for determination of oxygen content. Wedged liver capillary pressure was measured in most patients. In all but two patients the pulmonary artery was then again catheterized and a further double

† Dr. Parzer died from a cerebral tumor on September 11, 1955, at the age of 28. For all of us, it has been a privilege to know him and to work with him.

estimation of cardiac output was carried out. In the cardiac cases pulmonary artery, wedged pulmonary capillary, right atrial, and brachial or femoral artery pressures were measured using two Tybjaerg-Hansen manometers. Since the data on cardiac output and hepatic oxygen consumption and the pressure data have no bearing on the matter under discussion they will be omitted from this paper.

Bromsulphalein. "Bromthalein"-Merck was used throughout this study. Each patient was first given a single dose of 5 mgm. per Kg. body weight and its disappearance from the circulation was followed for 45 minutes. Three to 5 days later, catheterization was performed and immediately following a priming dose of 150 mgm., a constant i.v. infusion was started using a motor-driven syringe at a rate which, varying between 1 and 7 mgm. per min. in individual cases, was adjusted to the removal rate estimated from the clearance of the single BSP dose. Starting 10 min. later blood samples were taken at frequent intervals from the hepatic vein and from either the brachial or the femoral artery. A Zeiss-Opton photoelectric spectrophotometer was used to measure dye concentrations at a wave-length of 5800 Å. By plotting the concentration values on semi-log paper the rate of removal of BSP from the circulation was calculated from the infusion rate and from the change in peripheral BSP concentration per min., multiplied by the plasma volume. Hepatic blood flow (EHBF) and BSP extraction by the liver (E_{BSP}) was calculated as described by Bradley, Ingelfinger, Bradley, and Curry (2).

Colloidal radiogold. External measurements of the disappearance rate of colloidal Au^{198} from the blood stream were done during the time of BSP infusion. The procedure was carried out as previously described (1) with the exception that a shielded scintillation counter fitted with a $1\frac{1}{2} \times 1$ inch thallium-activated sodium iodide crystal was used instead of the lead cathode GM-counter, the opening of the shielding placed close to the skin over one of the thighs. A counting rate meter was used to read off counts per second at intervals of 1 min. and recently an ink-writer was attached to enable automatic recording. Colloidal Au^{198} produced by A.E.R.E., Harwell, now obtainable with a specific activity of up to 100 mc. per ml., was again used. It might be worthwhile to note that according to Lahr, Olsen, Gleason, and Tabern (3), the American-produced "Aurcoloid" has an average particle size of only about one-fifth of that of the British-made colloidal radiogold and might, therefore, be unsuitable for this type of experiment.

Blood volume. Whole body hematocrit and plasma volume were determined and blood volume was calculated as previously described (1) with the exception that for determination of plasma volume, instead of T-1824, human serum albumin labeled with I^{132} was used in a few cases.

RESULTS

It will be seen from the data presented in Table I that hepatic blood flow values estimated by the radiogold method (LMV) were, on the average,

TABLE I

Data on liver blood flow and BSP extraction estimated by the radiogold and by the BSP method in 15 subjects with varying degrees of hepatic dysfunction *

Name	Diagnosis	WBHT	BV	k	LMV	A	H	R	EHBF	E_{BSP}	LMV/EHBF
TOM	Cirrhosis	43.1	4,410	.0815	360	2.03	1.49	2.38	785	26.4	.459
HOF	Posthepatitis	34.6	5,550	.182	1,010	1.24	.66	6.50	1,715	46.8	.590
WEIN	Posthepatitis	32.4	4,220	.159	670	2.52	1.93	4.48	1,120	23.4	.598
SIM	Mitral stenosis	34.8	4,310	.071	305	4.62	2.26	7.37	480	51.0	.635
POL	Mitral stenosis	35.0	3,880	.115	445	2.50	1.41	4.59	645	43.6	.690
STE	Pericarditis	40.3	5,010	.1415	710	2.27	1.44	5.06	1,025	36.4	.693
MAI	Colitis	32.9	5,700	.128	730	.96	.24	5.00	1,035	75.0	.705
ZEC	Lues latens	29.0	5,750	.124	715	1.32	.57	4.79	900	56.8	.795
BOG	Appendicitis	37.2	6,050	.141	855	1.03	.10	6.00	1,030	90.5	.830
WAW†	Ductus botalli	36.7	4,520	.289	1,305				1,460		.895
WEIH	Posthepatitis	39.2	5,280	.161	850	2.28	.82	7.53	850	64.0	1.000
OBE	Mitral stenosis	39.3	5,290	.1135	600	1.18	.10	3.86	595	91.5	1.010
SIK	Diabetes	29.8	5,240	.231	1,210	.78	.10	5.00	1,050	87.2	1.151
NED	Postcholangitis	28.8	5,380	.180	970	.66	.37	1.56	750	44.0	1.291
KLI	Cirrhosis	36.3	5,990	.136	815	1.33	.91	1.67	620	31.6	1.313
Mean											.844

* WBHT = Whole body hematocrit, in per cent.
BV = Blood volume, corrected for blood loss during experiment, in ml.
k = Disappearance rate constant of Au^{198}, in $min.^{-1}$
LMV = Liver blood flow, calculated from BV and k, in ml. per min.
A = Peripheral BSP concentration, in mgm. per cent.
H = Hepatic vein BSP concentration, in mgm. per cent.
R = BSP removal rate, in mgm. per min.
EHBF = Liver blood flow, calculated from R, WBHT, and A-H difference, in ml. per min.
E_{BSP} = BSP extraction, calculated from A and A-H difference, in per cent.
† Detailed BSP data lost after completion of EHBF calculation.

84.4 per cent of those obtained by the BSP method (EHBF). There was considerable scatter around this mean, the factor LMV over EHBF ranging from 0.459 to 1.313. In four cases LMV was found to exceed EHBF, in one case both values were identical, and in the remaining 10 cases LMV was lower than EHBF (Figure 1). In most patients, liver blood flow values calculated by either method were found to be lower than normal.

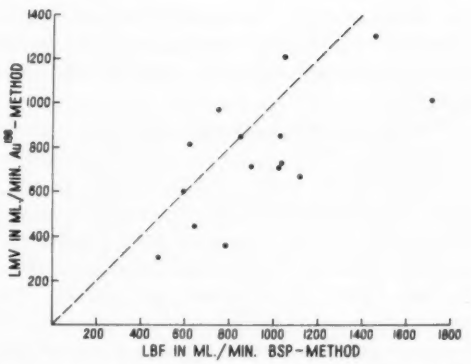

FIG. 1. COMPARISON OF LIVER BLOOD FLOW VALUES OBTAINED BY THE RADIOGOLD AND BY THE BROMSULPHALEIN METHOD

The dashed line represents a 1:1 relation.

DISCUSSION

The average difference of about 16 per cent between the radiogold and the BSP values could easily be explained by the fact that the radiogold method by neglecting incomplete removal of the colloid material by the liver phagocytes underestimates true hepatic blood flow. However, the large individual scatter and the observation in four cases of an EHBF which was lower than the radiogold value, appear to indicate that either the radiogold or the BSP method or both may be subject to considerable errors.

The nature and the magnitude of the errors involved in the radiogold method have been, up to now, not explored adequately. The results of some preliminary experiments permit only tentative statements which might, in the light of further observation, require drastic revision:

1. Though it was found that, on the average, 18 per cent of the large and medium-sized colloid particles are not removed by the liver phagocytes during their first passage through the liver capillaries, this figure is based on but 3 measurements of radiogold concentration in hepatic vein blood (1). Though published results obtained in animals (4, 5) suggest that this average is probably of the correct order, the magnitude of the individual variations around this mean needs to be established by further work.

2. The radiogold method is based on the assumption that mixing of the injected colloid within the circulating blood volume is very rapid. That this is not always the case has been shown by Dobson, Warner, Finney, and Johnston (6) and by Neumayr, Falkner, and Vetter (7) by comparison with the simultaneously observed mixing curve of T-1824. It could be demonstrated that, on the average, the influence of incomplete mixing can be neglected (7); however, an occasional delay in mixing could have contributed to the observed variations in individual differences between the calculated flow values.

3. The results of experiments on the distribution of intravenously injected colloidal radiogold in animals (3, 8, 9) indicate that small fractions of the administered dose are picked up by the adrenals, the kidneys, and the bone marrow. In postmortem studies of human subjects given therapeutic doses of the British radiogold intravenously, between 90 and 95 per cent could be accounted for in liver and spleen (10). It is probable that the remaining fractions are formed by the very small particles which pass the liver and spleen anyway, and whose disappearance curve from the circulation has to be separated graphically from the disappearance curve of the large and medium-sized particles. The error arising from this source should, therefore, be small; moreover, the removal rate of the small particles appears to be several times slower than that of the larger particles (7).

4. The nature of the colloid used is apparently of no importance provided the particle size does not differ greatly. Similar removal rates for different colloids with the exception of yttrium compounds have been observed in various animals (4, 11–13). In this laboratory, colloidal iron saccharate labeled with Fe^{59} has been observed to disappear from the human circulation at rates which did not significantly differ from the disappearance rates of colloidal radiogold (14).

5. If the amount of colloidal radiogold injected is kept below 250 μgm., "saturation" of the uptake mechanisms in the liver and spleen is unlikely to occur (1).

The nature and magnitude of the errors involved in the BSP method are far better known. Throughout this study all efforts were made to follow the procedures of infusing, timing of sample withdrawal, and of measuring BSP plasma concentration described by Bradley, Ingelfinger, Bradley, and Curry (2) as closely as possible. The results of several experiments had to be discarded because either the change in peripheral BSP concentration was too rapid or BSP extraction by the liver was too small, but even in the remaining cases considerable difficulties were encountered in the calculation of EHBF from the BSP data. Though these difficulties may have been aggravated to some extent by the inexperience of the authors with this method, some of the errors appeared to be unavoidable:

1. In most cases a steady state between BSP infusion and BSP removal could not be reached and BSP extraction by the liver decreased steadily during the experiment. Since this decrease was not accompanied by an immediate and corresponding increase in peripheral BSP concentration the calculated EHBF figures rose steadily. Though in some cases hepatic blood flow might have indeed changed it is improbable that it had changed in the same direction and to such an extent in so many cases. Moreover, cardiac output more often decreased than increased during the experiment. Thus, it was quite difficult to decide which of the several EHBF and E_{BSP} values which could be calculated during the period from 10 to 45 min. after starting the infusion, should be taken to come closest to the correct value. As a routine procedure that value was taken which was calculated from a period of time during which peripheral BSP concentration remained constant or nearly so, usually the 25 to 30-min. period. Though in these subjects the increase in peripheral BSP concentration which should follow a fall in BSP extraction in case the flow remains constant, was usually delayed by 5 to 10 min., the authors believe that the fall in calculated E_{BSP} figures was caused by a progressive impairment of the ability of the liver to remove BSP from the circulation. The same mechanism has been shown by Mendeloff, Kramer, Ingelfinger, and Bradley (15) to be responsible for the so-called "saturation effect" which is frequently observed after a single intravenous BSP dose. Even in normal cases the rate of BSP excretion into the bile is consistently below the rate of BSP removal by the liver parenchyma (16, 17); since the limiting factor would be the volume of the BSP space within the liver, after some time following injection the removal rate must slow down to the rate of biliary excretion. In cases with hepatic dysfunction it is likely that the biliary excretion rate as well as the volume of the BSP space is decreased and, in fact, the "saturation effect" has been observed more frequently in patients with hepatic disorders (18–20). In the present series the "saturation effect" has been seen after a single BSP dose in five cases. The observation that in these cases the differences between the radiogold and the BSP flow values were, on the average, 31 per cent whereas in the cases without "saturation" the average difference was only 8 per cent would indicate that the progressive impairment of BSP extraction by the liver might have contributed to inaccuracies in the estimation of EHBF.

2. Neither a rise of the peripheral BSP level over 4 mgm. per cent nor a fall below 1 mgm. per cent appeared to have been a major source of error, the differences between the calculated flows being evenly distributed over the whole range of peripheral BSP concentrations.

3. The original work on the BSP method was based on the assumption that BSP is removed from the circulation exclusively by the liver (2). However, it has been recently shown by Brauer, Pessotti, and Krebs (21) that in dogs after prolonged infusion at least 20 per cent of the infused BSP dose is found in other organs, especially the skeletal musculature. In animals in which hepatic dysfunction was produced by pretreatment with CCl_4, this figure was even considerably higher. As long as it is not firmly established that the extrahepatic BSP removal rates are several times slower than the hepatic removal rate it must be assumed that this effect leads to an overestimation of the true hepatic blood flow.

4. Entero-hepatic recirculation of BSP might have caused small inaccuracies in the calculated EHBF values.

5. A blood sample drawn from a single hepatic vein is not representative of all the blood leaving the liver. BSP concentrations in samples withdrawn at different locations of the catheter tip in the same liver may show considerable differences in extraction of BSP by different parts of the liver, and hepatic blood flow values calculated therefrom were found to vary around the mean of all values by up to 25 per cent (*2*); however, it can be assumed that the magnitude of these variations is usually smaller than this maximal value.

6. In restless patients it is sometimes difficult to maintain the tip of the catheter in exactly the same position, and hepatic vein blood may be inadvertently contaminated with inferior vena cava blood. By frequent fluoroscopic controls rigorous attention has to be paid to continually proper placement of the catheter.

Both methods depend on the accuracy of the blood volume determination. In the present study the calculation of both liver blood flow values has been based on the same plasma volume and the same whole body hematocrit; therefore, any error in the estimation of these values cannot be made responsible for the individual differences between the liver blood flow values.

The majority of the known errors of the BSP method appear to lead to an overestimation of the true hepatic blood flow and this has been shown to be true by comparison with direct blood flow determinations performed by Selkurt (*22, 23*). From the limited information now available it is most probable that the radiogold method, on the other hand, underestimates the true hepatic blood flow and it seems only surprising that, on the average, the differences between both values observed in the present study have not been greater.

The large individual variations in the direction and the magnitude of the differences could be explained by delays in the assumed rapid mixing of the injected radiogold within the circulation and by variations in the efficiency of splenic and hepatic removal of the colloid material as well as by nonuniform extraction of BSP by the liver.

SUMMARY

1. In 15 patients with hepatic or cardiac disorders hepatic blood flow was estimated simultaneously by the bromsulphalein and by the radiogold method.

2. Liver blood flows calculated from the disappearance rate constant of colloidal Au^{198} and from the blood volume, were found to average 84.4 per cent of the values obtained by the bromsulphalein method, with large individual variations in the direction and in the magnitude of the differences.

3. The source and magnitude of the errors involved in both methods which may have been responsible for these differences are discussed.

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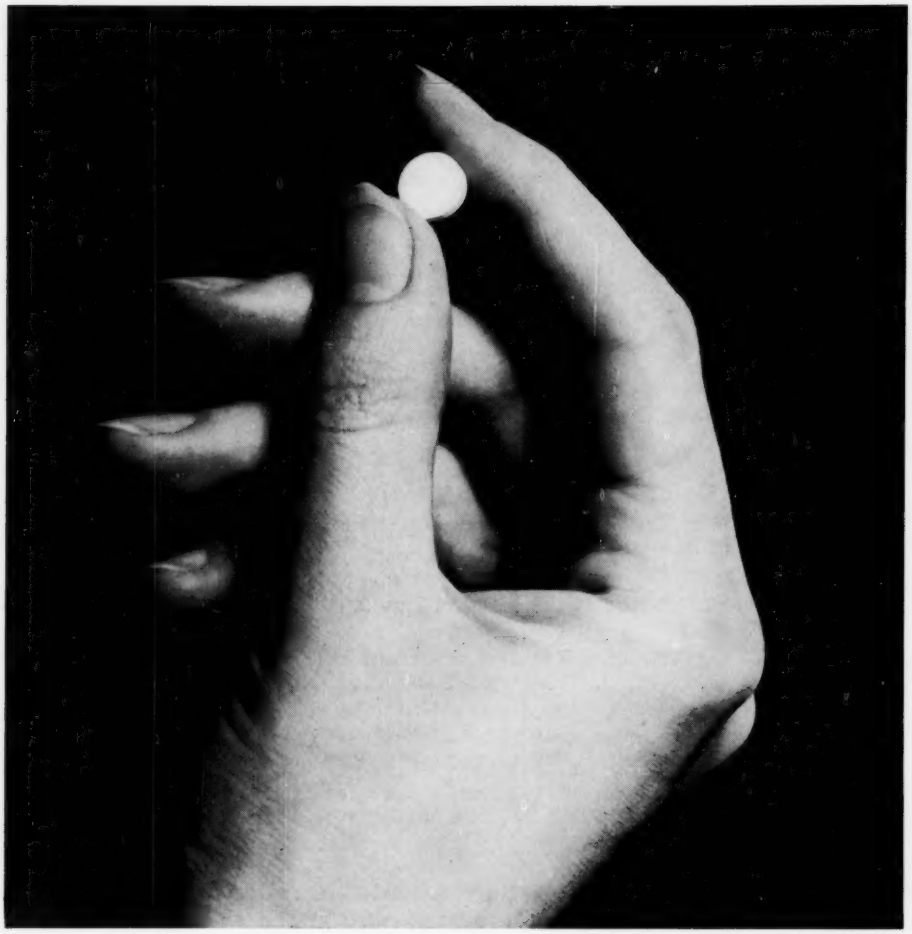

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A bacteriostatically-inert aryl sulfonylurea that produces hypoglycemia in intact animals but not in depancreatized or alloxan-diabetic animals shows promise in the treatment of certain types of diabetes mellitus.

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Preliminary observations show that this compound is orally effective in reducing hyperglycemia and glycosuria in patients with the adult form of diabetes mellitus, but probably will not replace insulin in the treatment of severe diabetes, diabetes of childhood, or diabetes complicated by acidosis or infection. The mechanism of this hypoglycemic action remains to be clarified.

While early clinical studies are encouraging, the decision to market Orinase must await substantiation and verification by careful detailed investigation. Such work is now in progress, but may take many months to complete.

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